CNG2018 022

POLIMORFISMOS NULOS DE LOS GENES *GSTT1* Y *GSTM1* Y ENFERMEDAD DE PARKINSON

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La enfermedad de Parkinson (EP) es una patología neurodegenerativa que afecta aproximadamente al 3% de la población mundial, es multifactorial. Diversos estudios han asociado factores genéticos y ambientales con el desarrollo de la EP. Las Glutation S Transferasa (GST) son una familia de enzimas que intervienen tanto en el metabolismo de toxinas como en la desintoxicación celular lo que hipotéticamente implica una función neuroprotectora. Las GST más ampliamente estudiadas en relación a la EP son la GSTM1 y la GSTT1. Aunque se ha sugerido una asociación entre mutaciones nulas en estos genes y la EP, también hay estudios que sugieren que no existe, por lo que se ha propuesto que dicha asociación dependería de la población analizada. Por tal motivo, el objetivo de este trabajo fue determinar si existe una asociación entre las mutaciones nulas en *GSTT1* y *GSTM1* y la EP en población mexicana. Se llevó a cabo un estudio de casos (75 pacientes con diagnóstico de EP) y testigos (75 individuos sin enfermedad neurodegenerativa) los cuales fueron pareados por edad y género. Se obtuvo DNA de sangre periférica y se realizó la genotipificación por PCR de punto final. Se realizaron las pruebas UPDRS, minimental y Hamilton para evaluar la severidad de la EP, estado cognitivo y depresión, respectivamente. La media de edad tanto para casos como para testigos fue de 70 años. Al comparar los resultados de las pruebas de minimental y Hamilton entre casos y testigos, solo la escala de Hamilton presentó diferencias estadísticamente significativas ($p<0.001$), siendo mayor en los casos que en los testigos. La media para los casos del UPDRS fue de 66.63. La mutación nula *GSTT1* se presentó en 7 de los casos y 11 de los testigos y la mutación nula en *GSTM1* en 27 de los casos y 26 de los testigos. Al comparar ambos grupos no encontramos diferencias estadísticamente significativas ni para la mutación nula en *GSTT1* ni para *GSTM1* ($p=0.314$ y $p=0.864$, respectivamente). En conclusión, los resultados sugieren que no existe asociación entre las mutaciones nulas en *GSTT1* y *GSTM1* y la EP.

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XLII Congreso Nacional de Genética Humana

MÉRIDA, YUCATÁN, Noviembre 29 a Diciembre 2 de 2017

La Asociación Mexicana de Genética Humana

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Elizabeth I. Antuna-Salcido, Cosme Alvarado-Esquivel, Edna M. Méndez-Hernández, Jesús Hernández-Tinoco, Luís F. Sánchez-Anguiano, Francisco X. Castellanos-Juárez, Osmel La Llave-León, José M. Salas-Pacheco y Ada Sandoval-Carrillo

Por la presentación del trabajo libre en modalidad Cartel:

VARIANTES DEL GEN *TNF-α* Y SU ASOCIACIÓN CON DEPRESIÓN EN MUJERES EMBARAZADAS

Mérida, Yucatán, diciembre 1 de 2017

DR. JORGE E. ZAVALA CASTRO
Director CIR Dr. Hideyo Noguchi UADY

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Presidente AMGH

DR. RODRIGO RUBI CASTELLANOS
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INSTITUTO DE INVESTIGACIÓN CIENTÍFICA

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Por la presentación del trabajo ***"VARIANTES DEL GEN TNF-α Y SU ASOCIACIÓN CON DEPRESIÓN EN MUJERES EMBARAZADAS"****, realizado en las Jornadas Académicas "La Investigación Científica, Compromiso y Pertinencia Social", en el marco conmemorativo del XLVIII Aniversario del IIC y II Encuentro de Investigación de la DES - Ciencias de la Salud de la UJED.*

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Dr. Luis Francisco Sánchez Anguiano
Director del IIC

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CNG2018 025

CARACTERIZACION DE LAS VARIANTES rs1805386 del gen *LIG4* y rs1805377 del gen *XRCC4* y SU ASOCIACIÓN CON LA PREECLAMPSIA

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La preeclampsia (PE), enfermedad exclusiva del embarazo, es una de las principales causas de mortalidad materna en el mundo. Se caracteriza por presión arterial mayor de 140/90 mm/Hg y proteinuria mayor de 0,3 g/l después de las 20 semanas de gestación. Hoy en día se reconoce a la preeclampsia como un desorden placentario que tiene un origen genético multifactorial, es decir, es resultado de la interacción de genes y factores ambientales. A la fecha existen diversos estudios que demuestran que el daño al ADN es más elevado en pacientes con PE. Debido a esto, se ha propuesto que variantes en genes que participan en los procesos de reparación del ADN pueden asociarse a la PE. Por lo antes mencionado, el objetivo principal del presente trabajo fue determinar la asociación entre las variantes rs1805386 del gen *LIG4* y rs1805377 del gen *XRCC4* y la PE en mujeres de Durango. Se llevó a cabo un estudio transversal, observacional de casos y controles. Se incluyeron 155 mujeres con PE y 160 con embarazo normotenso. La genotipificación se realizó mediante PCR en tiempo real. Los controles presentaron una media de edad de 24.52±7.32 años y los casos de 23.53±6.8 años (p=0.083). Las medias de semanas de gestación fueron 37.95±3.54 y 35.38±5.30 para los controles y casos, respectivamente (p<0.001). El 30% de los controles y el 43.2% de los casos tuvo antecedentes de PE (p=0.015). Las frecuencias alélicas y genotípicas para la variante rs1805386 fueron T=0.90, C=0.10, T/T=0.84, T/C=0.11 y C/C=0.05 para los controles y T=0.93, C=0.07, T/T=0.85, T/C=0.14 y C/C=0.01 para los casos. Para la variante rs1805377 fueron G=0.62, A=0.38, G/G=0.37, G/A=0.49 y A/A=0.14 para los controles y G=0.6, A=0.4, G/G=0.4, G/A=0.4 y A/A=0.2 para los casos. No encontramos diferencias estadísticamente significativas para ninguna de las variantes al, comparar los grupos. Finalmente, se estimó la OR ajustando por edad y semanas de gestación; no encontramos asociación entre las variantes y la PE. En conclusión, nuestros resultados sugieren que en nuestra población, las variantes rs1805386 del gen *LIG4* y rs1805377 del gen *XRCC4* no se asocian con la PE.

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II Jornada Nacional de Investigación en Salud

DURANGO 2018

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la Secretaría de Salud

Otorga la presente

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a: **La Llave León O., Castellanos Juárez F., Méndez Hernández E., Sandoval Carrillo A., Esquivel Rodríguez E., García Vargas G., Duarte Sustaita J., Salas Pacheco JM.**

Por su participación como **Ponente** dentro de la

II Jornada Nacional de Investigación en Salud Durango 2018

Con el tema:

Niveles de Plomo en Sangre y su Asociación con la Actividad de la Enzima Ácido Delta-Aminolevulínico Deshidratasa

Habiendo obtenido el **Tercer Lugar** en la Categoría de **Investigación en Salud Pública**

los días 18, 19 y 20 de octubre del 2018, en el
Centro Cultural y de Convenciones Bicentenario, Durango, Dgo.

Victoria de Durango, Dgo. octubre de 2018

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A ***Osmel La Llave León, Francisco X. Castellanos Juárez, Edna Méndez Hernández, Ada Sandoval Carrillo, Eloísa Esquivel Rodríguez, Gonzalo García Vargas, Jaime Duarte Sustaita, José M. Salas Pacheco***

Por la presentación del trabajo ***"ASOCIACIÓN ENTRE LOS NIVELES DE PLOMO EN SANGRE Y LA ACTIVIDAD DE LA ENZIMA ÁCIDO DELTA-AMINOLEVULÍNICO DESHIDRATASA"****, realizado en las Jornadas Académicas "La Investigación Científica, Compromiso y Pertinencia Social", en el marco conmemorativo del XLVIII Aniversario del IIC y II Encuentro de Investigación de la DES - Ciencias de la Salud de la UJED.*

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Por la presentación del trabajo ***"RELACIÓN ENTRE LOS NIVELES DE PLOMO EN SANGRE Y LA EXPOSICIÓN OCUPACIONAL EN MUJERES EMBARAZADAS DE DURANGO"****, realizado en las Jornadas Académicas "La Investigación Científica, Compromiso y Pertinencia Social", en el marco conmemorativo del XLVIII Aniversario del IIC y II Encuentro de Investigación de la DES - Ciencias de la Salud de la UJED.*

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UNIVERSIDAD AUTÓNOMA DE YUCATÁN
MÉRIDA

Edith Maldonado-Soto, Sergio M. Salas-Pacheco, Ernesto G. Miranda-Morales, Edna M. Méndez-Hernández, Francisco X. Castellanos-Juárez, Osmel La Llave-León, Oscar Arias-Carrión, José M. Salas-Pacheco y Ada Sandoval-Carrillo

Por la presentación del trabajo libre en modalidad Cartel:

POLIMORFISMOS EN GENES DE REPARACION DE ADN Y SU ASOCIACIÓN CON LA ENFERMEDAD DE PARKINSON

Mérida, Yucatán, noviembre 30 de 2017

DR. JORGE E. ZAVALA CASTRO
Director CIR Dr. Hideyo Noguchi UADY

DRA. DORIS PINTO ESCALANTE
Presidente AMGH

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Por la presentación del trabajo ***"POLIMORFISMOS EN GENES DE REPARACION DE ADN Y SU ASOCIACIÓN CON LA ENFERMEDAD DE PARKINSON"****, realizado en las Jornadas Académicas "La Investigación Científica, Compromiso y Pertinencia Social", en el marco conmemorativo del XLVIII Aniversario del IIC y II Encuentro de Investigación de la DES - Ciencias de la Salud de la UJED.*

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Dr. Luis Francisco Sánchez Anguiano
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JN-CAR-IC-44.

PARAMETROS HEMATOLÓGICOS EN SUJETOS DURANGUENSES CON ENFERMEDAD DE PARKINSON

Miranda Morales Ernesto Gerardo[1], Castellanos Juárez Francisco Xavier[1], La Llave León Osmel[1], Méndez Hernández Edna Madai[1], Sandoval Carrillo Ada[1], Quiñones Canales Gerardo[2], Ruano Calderón Luis Ángel[3], Arias Carrión Oscar4 y Salas Pacheco José Manuel[1].

1. Instituto de Investigación Científica, UJED-Durango
2. Hospital General Santiago Ramón y Cajal-ISSSTE, Durango
3. Hospital General 450, Durango
4. Hospital General Dr. Manuel Gea González, Ciudad de México

INTRODUCCIÓN.

Los parámetros hematológicos y su asociación con la Enfermedad de Parkinson (EP) han sido poco descritos. En particular, el incremento en los niveles de Hemoglobina (Hb) se ha asociado con una mayor incidencia de EP.

OBJETIVO.

Determinar si existen diferencias en los parámetros hematológicos entre sujetos con EP y un grupo control.

MATERIALES Y MÉTODOS.

Estudio de 35 casos de EP y 35 controles en sujetos que acudieron al Hospital General 450, el Hospital Santiago Ramón y Cajal del ISSSTE y Ciudad del Anciano en Durango.

RESULTADOS.

Los niveles de Hb fueron de 14.71±2.02 dL y 14.21±2.02 dL, en casos y controles, respectivamente (p = 0.315). El recuento de glóbulos rojos (RBC) de casos fue de 4.72±0.51 y de los controles de 4.74±0.63 (p = 0.939). El volumen corpuscular medio (VCM) de 90.72±7.73 en casos y 91.62±4.98 en controles (p = 0.264). El Hematocrito (HCT) se encontró en 42.87±5.75 en casos y 43.35±5.54 en controles (p = 0.485). La Hemoglobina corpuscular media (HCM) fue de 31.08±2.11 en casos y 30.05±2.20 en controles (p = 0.049). Finalmente, la concentración de la hemoglobina corpuscular media (CHCM) se encontró en 34.35±2.04 en casos y 32.79±1.81 en controles (p < 0.001).

CONCLUSIÓN.

Aunque los niveles de Hb fueron ligeramente mayores en los casos, no se encontraron diferencias significativas. Sin embargo, si se observaron diferencias significativas en los niveles de HCM y CHCM. Futuros estudios con tamaños muestrales mayores son necesarios para corroborar estos hallazgos en nuestra población.

PALABRAS CLAVE.

Enfermedad de Parkinson, parámetros hematológicos, hemoglobina.

SALUD CON CIENCIA

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JN-CAR-IC-45.

NIVELES DE ÁCIDO ÚRICO EN SUJETOS DE DURANGO CON ENFERMEDAD DE PARKINSON

Miranda Morales Ernesto Gerardo[1], Castellanos Juárez Francisco Xavier[1], La Llave León Osmel[1], Méndez Hernández Edna Madai[1], Sandoval Carrillo Ada[1], Quiñones Canales Gerardo[2], Ruano Calderón Luis Ángel[3], Arias Carrión Oscar[4], Salas Pacheco José Manuel[1].

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3. Hospital General 450. Durango
4 Hospital General Dr. Manuel Gea González, Ciudad de México

INTRODUCCIÓN.

La función del ácido úrico (AU) y su efecto fisiopatológico en casos de EP en México han sido poco descritos. El AU ejerce un efecto antioxidante en neuronas y se conoce como un quelador de hierro. Previos reportes en otras poblaciones han asociado niveles bajos de AU con la EP.

OBJETIVO.

Determinar si existen diferencias en los niveles séricos de AU entre casos de EP y controles en población Duranguense.

MATERIAL Y MÉTODO.

Estudio de 61 casos de EP y 69 controles en sujetos que acudieron al Hospital General 450, el Hospital Santiago Ramón y Cajal del ISSSTE y Ciudad del Anciano en Durango.

RESULTADOS.

Encontramos niveles de AU de 5.35 $\pm$ 2.30 mg/dL para los casos y 6.03 $\pm$ 1.31 mg/dL para los controles (p = 0.010). Al estratificar por género, los niéveles de AU en mujeres con EP fue de 5.35 $\pm$ 1.46 mg/dL y de 5.62 $\pm$ 1.24 mg/dL para el grupo control (p = 0.442). En el grupo de hombres los niveles de AU fueron de 5.52 $\pm$ 6.45 mg/dL para los casos con EP y 6.46 $\pm$ 1.26 mg/dL para los controles (p = 0.008).

CONCLUSIONES.

Existen diferencias estadísticamente significativas en los niveles de AU, siendo menores en los individuos con EP. Al estratificar por género, observamos que esta diferencia solamente se mantiene en los hombres. Nuestros resultados concuerdan con lo previamente reportado en otras poblaciones.

PALABRAS CLAVE.

Enfermedad de Parkinson, ácido úrico, antioxidante.

SALUD CON CIENCIA

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JN-CAR-IC-46.

CARACTERIZACIÓN DE LAS VARIANTES H1/H2 DE MAPT Y RS1801133 DE MTHFR EN SUJETOS MEXICANOS CON ENFERMEDAD DE PARKINSON

Miranda Morales Ernesto Gerardo[1], Castellanos Juárez Francisco Xavier[1], La Llave León Osmel[1], Méndez Hernández Edna Madai[1], Sandoval Carrillo Ada[1], Quiñones Canales Gerardo[2], Ruano Calderón Luis Ángel[3], Arias Carrión Oscar[4], Salas Pacheco José Manuel[1].

1. Instituto de Investigación Científica, UJED-Durango
2. Hospital General Santiago Ramón y Cajal-ISSSTE, Durango
3. Hospital General 450, Durango
4. Hospital General Dr. Manuel Gea González, Ciudad de México

INTRODUCCIÓN.

Se han identificado mutaciones y polimorfismos en genes relacionados con la Enfermedad de Parkinson (EP). No obstante, las bases genéticas, y bioquímicas asociadas a la EP han sido poco estudiadas en nuestro país.

OBJETIVO.

Genotipificar los haplotipos H1/H2 de MAPT y rs1801133 del gen MTHFR en sujetos con EP e individuos sanos. Posteriormente, se determinará si estos polimorfismos están asociados a cambios epigenéticos.

MATERIAL Y MÉTODO.

Estudio de 108 casos y 91 controles en sujetos que acudieron al Hospital General Dr. Manuel Gea González en la Ciudad de México, el Hospital General 450, el Hospital Santiago Ramón y Cajal del ISSSTE y Ciudad del Anciano en Durango.

RESULTADOS.

Las frecuencias para los genotipos H1/H2 de MAPT fueron H1/H1: 0.80, H1/H2: 0.18 y H2/H2: 0.02, con respecto a los casos y H1/H1: 0.85, H1/H2: 0.14, y H2/H2: 0.01, con respecto a los controles. Las frecuencias genotípicas para el SNP rs1801133 de MTHFR fueron C/C: 0.19, C/T: 0.47, y T/T: 0.34, con respecto a los casos y C/C: 0.23, C/T: 0.53, y T/T: 0.24, para los controles. No se observaron diferencias estadísticamente significativas al comparar las frecuencias alélicas y genotípicas entre los casos y los controles (p = 0.3600 para H1/H2 de MAPT y p = 0.1450 para rs1801133 de MTHFR). Al estratificar por región (centro y norte del país) tampoco se observaron diferencias.

CONCLUSIONES.

Nuestros resultados sugieren que las variantes estudiadas no se asocian con la EP en la población estudiada.

PALABRAS CLAVE.

Enfermedad de Parkinson, MAPT, MTHFR.

SALUD CON CIENCIA

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Por la presentación del trabajo ***"PARÁMETROS HEMATOLÓGICOS EN SUJETOS DE DURANGO CON ENFERMEDAD DE PARKINSON"****, realizado en las Jornadas Académicas "La Investigación Científica, Compromiso y Pertinencia Social", en el marco conmemorativo del XLVIII Aniversario del IIC y II Encuentro de Investigación de la DES - Ciencias de la Salud de la UJED.*

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A ***Miranda Morales Ernesto Gerardo, Castellanos Juárez Francisco Xavier, La Llave León Osmel, Méndez Hernández Edna Madai, Sandoval Carrillo Ada, Quiñones Canales Gerardo, Ruano Calderón Luis Ángel, Arias Carrión Oscar y Salas Pacheco José Manuel***

Por la presentación del trabajo ***"CARACTERIZACIÓN DE LAS VARIANTES H1/H2 DE MAPT y rs1801133 DE MTHFR EN SUJETOS MEXICANOS CON ENFERMEDAD DE PARKINSON"****, realizado en las Jornadas Académicas "La Investigación Científica, Compromiso y Pertinencia Social", en el marco conmemorativo del XLVIII Aniversario del IIC y II Encuentro de Investigación de la DES - Ciencias de la Salud de la UJED.*

Atentamente

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Victoria de Durango, Dgo. a 05 de Octubre de 2018

Dr. Luis Francisco Sánchez Anguiano
Director del IIC

Dra. Laura Ernestina Barragán Ledesma
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Ciencias de la Salud

UNIVERSIDAD JUÁREZ DEL ESTADO DE DURANGO
INSTITUTO DE INVESTIGACIÓN CIENTÍFICA

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Por la presentación del trabajo ***"NIVELES DE ÁCIDO ÚRICO EN SUJETOS DE DURANGO CON ENFERMEDAD DE PARKINSON"****, realizado en las Jornadas Académicas "La Investigación Científica, Compromiso y Pertinencia Social", en el marco conmemorativo del XLVIII Aniversario del IIC y II Encuentro de Investigación de la DES - Ciencias de la Salud de la UJED.*

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XLII Congreso Nacional de Genética Humana

MÉRIDA, YUCATÁN, Noviembre 29 a Diciembre 2 de 2017

La Asociación Mexicana de Genética Humana

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ASOCIACIÓN MEXICANA DE GENÉTICA HUMANA A.C.

CENTRO DE INVESTIGACIONES REGIONALES "DR. HIDEYO NOGUCHI"
UNIVERSIDAD AUTÓNOMA DE YUCATÁN
MÉXICO

Alma Cristina Salas Leal, Francisco Xavier Castellanos Juárez, Osmel La Llave León, Edna Madai Méndez Hernández, Gerardo Quiñones Canales, Luis Ángel Ruano Calderón, Oscar Arias Carrión, José Manuel Salas Pacheco y Ada Sandoval Carrillo

Por la presentación del trabajo libre en modalidad Cartel:

CARACTERIZACIÓN DE LOS PERFILES DE EXPRESIÓN DEL GEN *SNCA* Y SU VARIANTE rs356219 EN PACIENTES CON ENFERMEDAD DE PARKINSON

Mérida, Yucatán, diciembre 1 de 2017

DR. JORGE E. ZAVALA CASTRO
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DRA. DORIS PINTO ESCALANTE
Presidente AMGH

DR. RODRIGO RUBI CASTELLANOS
Secretario AMGH

JN-CAR-IC-47.

CARACTERIZACIÓN DE POLIMORFISMOS EN LOS GENES SNCA, UBE2K, ALDH1A1, HSPA8, SKP1A Y PSMC4 EN SUJETOS CON ENFERMEDAD DE PARKINSON

Salas Leal Alma Cristina[1], Castellanos Juárez Francisco Xavier[1], La Llave León Osmel[1], Méndez Hernández Edna Madai[1], Quiñones Canales Gerardo[2], Ruano Calderón Luis Ángel[3], Arias Carrión Oscar[4], Salas Pacheco José Manuel[1], Sandoval Carrillo Ada[1].

1. Instituto de Investigación Científica, UJED-Durango
2. Hospital General Santiago Ramón y Cajal-ISSSTE, Durango
3. Hospital General 450, Durango
4. Hospital General Dr. Manuel Gea González, Ciudad de México

INTRODUCCIÓN.

La enfermedad de Parkinson (EP) es una enfermedad neurodegenerativa que se manifiesta como una desregulación en el control del movimiento. La EP está caracterizada por la pérdida de dopamina y la presencia de cuerpos de Lewy, formados por ubiquitina y a-sinucleína. A la fecha se han realizado diversos estudios que han asociado variantes génicas con la EP en diversas poblaciones; sin embargo, este tipo de estudios son muy escasos en población mexicana.

OBJETIVO.

Determinar las frecuencias alélicas y genotípicas de los polimorfismos rs3764435 de ALDH1A1, rs234365 de PSMC4, rs2110585 de SKP1, rs305124 de UBE2K, rs2236659 de HSPA8 y rs356219 de SNCA y su asociación con la EP.

MATERIALES Y MÉTODOS.

Se reclutaron 45 casos y 70 controles. La genotipificación se realizó por PCR tiempo real. Los análisis se realizaron con el programa SNPStats.

RESULTADOS.

El análisis de las frecuencias alélicas y genotípicas evidenció que solo el polimorfismo rs356219 del gen SNCA es un factor de riesgo para la EP (OR=2.8, IC951.277-6.163, p=0.009). Las frecuencias alélicas para este polimorfismo fueron A=0.46, G=0.54 en controles y A=0.30, G=0.70, en casos. Las genotípicas fueron A/A=0.19, G/A=0.54, G/G=0.27 en controles y A/A=0.11, G/A=0.38, G/G=0.51 en casos.

CONCLUSIONES.

El alelo G del polimorfismo rs356219 del gen SNCA es más frecuente en los pacientes con EP. Nuestros resultados confirman lo reportado previamente en otras poblaciones en los que se ha observado que el alelo G incrementa el riesgo de la EP. No se observó asociación con ninguna de las otras variantes y la EP.

PALABRAS CLAVE.

Enfermedad de Parkinson, SNCA, rs356219.

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JN-CAR-IC-55.

CARACTERIZACIÓN DE LOS PERFILES DE EXPRESIÓN DEL GEN SNCA Y SU VARIANTE rs356219 EN PACIENTES CON ENFERMEDAD DE PARKINSON

Salas Leal Alma Cristina[1], Castellanos Juárez Francisco Xavier[1], La Llave León Osmel[1], Méndez Hernández Edna Madai[1], Quiñones Canales Gerardo[2], Ruano Calderón Luis Ángel[3], Arias Carrión Oscar[4], Salas Pacheco José Manuel[1], Sandoval Carrillo Ada[1].

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4. Hospital General Dr. Manuel Gea González, Ciudad de México

INTRODUCCIÓN.

La enfermedad de Parkinson (EP) es una enfermedad neurodegenerativa caracterizada por pérdida neuronal, disminución en la disponibilidad cerebral de dopamina y la presencia de cuerpos de Lewy. Estos están formados principalmente por la proteína a-sinucleína la cual es codificada por el gen SNCA. Los perfiles de expresión y variantes génicas han sido ampliamente estudiados en la EP en la búsqueda de posibles biomarcadores en sangre periférica.

OBJETIVO.

Determinar si el polimorfismo rs356219 modula los niveles de expresión del gen SNCA y si estos se asocian con la EP.

MATERIALES Y MÉTODOS.

Se reclutaron 15 casos y 15 controles pareados por edad y sexo. La cuantificación relativa de la expresión y la genotipificación se realizó por PCR tiempo real. Para el análisis estadístico se usaron pruebas para comparación de medias.

RESULTADOS.

Se obtuvieron expresiones similares del gen SNCA en individuos con EP con respecto a los controles sanos (0.751±0.32 vs 0.788±0.30; p=0.752). Los niveles de expresión en base al genotipo fueron 0.490±0.16 (A/A), 0.812±0.32 (A/G) y 0.72±0.17 (G/G). Al comparar en base a un modelo de herencia dominante, se observó una mayor expresión por la presencia de la variante alélica (A/A=0.490±0.16 vs A/G+G/G=0.788±0.29), con una tendencia a la significancia (p=0.057).

CONCLUSIONES.

No se observaron diferencias en los niveles de expresión entre los grupos lo cual concuerda con lo reportado por Tan y cols en 2005 en población china. Por otro lado, nuestros resultados sugieren que la presencia del alelo de riesgo se asocia con mayores niveles de expresión.

PALABRAS CLAVE.

Enfermedad de Parkinson, Perfiles de expresión, SNCA, rs356219

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CNG2018 007

ASOCIACIÓN DEL SNP RS3764435 DEL GEN ALDH1A1 CON ENFERMEDAD DE PARKINSON EN POBLACIÓN MEXICANA

Salas-Leal AC[1], Pérez-Gavilán Ceniceros JA[1], Salas-Pacheco JM[1], Arias-Carrión O[2], Quiñones-Canales G[3], Ruano-Calderón LA[4], Castellanos-Juárez FX[1], Mendez-Hernández EM[1], La Llave-León O[1], Sandoval-Carrillo AA[1]*

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La Enfermedad de Parkinson (EP) es el segundo desorden neurodegenerativo más frecuente. Recientemente se han reportado nuevos descubrimientos acerca de factores genéticos implicados en esta enfermedad. El gen ALDH1A1 codifica para la enzima aldehído deshidrogenasa, involucrada en la degradación de productos neurotóxicos resultado del metabolismo de la dopamina. Se ha demostrado que los niveles de ALDH1A1 y su actividad, se encuentran disminuidos en pacientes con EP. Entre los polimorfismos de un solo nucleótido (SNP) que podrían modular los niveles de expresión, se encuentra el SNP rs3764435 (A/C). El objetivo principal de este estudio fue establecer si existe asociación entre el SNP rs3764435 del gen ALDH1A1y la EP. Se trata de un estudio de casos (119 pacientes con diagnóstico de EP) y controles (177 individuos sin enfermedad neurodegenerativa). Se obtuvo ADN de sangre periférica y se realizó la genotipificaión por PCR tiempo real. El grupo control presentó una frecuencia para el alelo A=0.47 y para el alelo C=0.53; las frecuencias genotípicas fueron A/A=0.24, A/C=0.47 y C/C=0.29. Con respecto a los casos, las frecuencias alélicas fueron A=0.57 y C=0.43 y las genotípicas A/A=0.27, A/C=0.60 y C/C=0.13. Encontramos diferencias estadísticamente significativas entre los grupos tanto en las frecuencias alélicas como en las genotípicas (p=0.022 y p=0.006, respectivamente). El análisis de la estimación de riesgo evidenció que el genotipo C/C del SNP rs356219 del gen ALDH1A1 es un factor protector tanto en un modelo de herencia codominante como en el recesivo (OR=0.38, IC95%=0.20-0.71 y OR=0.42, IC95%=0.20-0.86, respectivamente). Nuestros resultados sugieren que el genotipo C/C del SNP rs3764435 del gen ALDH1A1 es un factor de protección para la EP en población mexicana y debido a su posición intrónica, se sugiere que el SNP puede tener un efecto positivo en la actividad enzimática como resultado del splicing alternativo o incluso influir en el incremento de la expresión génica.

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SOCIEDAD MEXICANA DE GENÉTICA

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Por la presentación del trabajo ***"α-SINUCLEINA Y ENFERMEDAD DE PARKINSON EN POBLACION MEXICANA",*** *realizado en las Jornadas Académicas "La Investigación Científica, Compromiso y Pertinencia Social", en el marco conmemorativo del XLVIII Aniversario del IIC y II Encuentro de Investigación de la DES - Ciencias de la Salud de la UJED.*

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REVISTA MEXICANA DE CIENCIAS FARMACÉUTICAS

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Huatulco - 4 al 7 de Septiembre 2016

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TRABAJO LIBRE - BDM00008

Valores de referencia de hemoglobina glucosilada de una población entre 20 y 30 años

Muñoz Estrada Marisol[1], Cortes Muñoz Verónica[1], García Jiménez Natividad Sara[1], Sánchez Alemán Miguel Ángel[2]

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Introducción: La diabetes mellitus tipo 2 (DM2) es una alteración metabólica, que se caracteriza por niveles elevados de glucosa, debido a una deficiencia en la producción o en la acción de la insulina; afecta a más de 11.5 millones de mexicanos en una edad entre 20 a 79 años. Es un padecimiento que favorece el desarrollo de complicaciones como retinopatías, nefropatías y enfermedades cardiovasculares. Existen criterios diagnósticos para la DM2, en donde a partir del 2010 la American Society of Diabetes (ADA) incorpora la hemoglobina glucosilada (HbA1c) como criterio de diagnóstico. La HbA1c es una proteína que sirve para estimar el promedio de glucosa de un paciente durante los últimos tres meses. Los valores de referencia, son magnitudes importantes que se utilizan para la interpretación médica de resultados clínicos y es fundamental que cada laboratorio de diagnóstico cuente con valores de referencia propios debido a que dependen de la etnia, la edad y el sexo.

Objetivo: Establecer valores de referencia de HbA1c en estudiantes de la Universidad Autónoma del Estado de Morelos (UAEM).

Metodología: Se realizó un estudio transversal con una muestra aleatoria de estudiantes de la UAEM. Los estudiantes interesados en participar firmaron una carta de consentimiento informado y contestaron un cuestionario socio-demográfico. Se realizarón mediciones antropométricas de peso, talla, circunferencia de cintura y presión arterial. Se tomó una muestra sanguínea para cuantificar los niveles de glucosa y de HbA1c. La determinación de HbA1c y glucosa se realizaron con técnicas inmunoenzimáticas con un equipo COBAS 111-Roche USA.

Resultados: Participaron 92 estudiantes entre 20 y 30 años de edad de las diferentes facultades la UAEM, obteniendo los siguientes resultados: 33% hombres y 59% mujeres, el valor promedio de glucosa en ayuno fue de 70.78 (±16.12) mg/dl, la media de la población de HbA1c fue de 5.41% (±0.232), y los intervalos de referencia obtenidos fueron: límite inferior de 4.94% y un límite superior de 5.88%

Conclusiones: Se obtuvo un intervalo de referencia para esta población de 4.94% a 5.88%, intervalo con una diferencia de 0.19%, pero cerca del valor reportado por la ADA y la OMS (< 5.7%) en personas sanas.

TRABAJO LIBRE - BDM00009

Genotipificación del polimorfismo rs2228001 del gen XPC en mujeres con preeclampsia y embarazo normoevolutivo

Salas Pacheco José Manuel, Ramírez Sosa Lino Enrique, Medina Simental Rosa Arlette, Castellanos Juarez Francisco Xavier, La Llave Leon Osmel, Méndez Hernández Edna Madai, Sandoval Carrillo Ada

INSTITUTO DE INVESTIGACIÓN CIENTÍFICA DE LA UJED

Introducción: La preeclampsia es un síndrome clínico caracterizado por hipertensión con disfunción orgánica múltiple, proteinuria y edema. Las bases moleculares de los factores causales implicados en la patogenia de la preeclampsia no son muy claras. Genes asociados con la prevención o reparación del ADN se han propuesto como candidatos potenciales para ser estudiados y determinar su posible rol en el desarrollo de la preeclampsia. El gen *XPC* codifica para una proteína indispensable del sistema de reparación por escisión de nucleótidos. El polimorfismo rs2228001 (T/G) ha sido asociado a distintos tipos de cáncer incluyendo el de pulmón y vejiga, sin embargo, no hay estudios que evalúen su posible asociación con la preeclampsia.

Objetivo: Determinar si existe asociación entre el polimorfismo rs2228001 del gen *XPC* y la preeclampsia.

Metodología: Estudio prospectivo de casos (diagnóstico de preeclampsia) y controles (embarazo normoevolutivo). De sangre periférica se extrajo ADN utilizando el sistema QIAamp DNA Blood Mini Kit. La genotipificación se realizó utilizando sondas Taqman. Se usaron medidas de tendencia central y de dispersión para los datos descriptivos, para las diferencias entre grupos se usaron las pruebas T de Student y Chi cuadrada. Para la estimación del riesgo (Odds Ratio, OR) se utilizó el software SNPstats.

Resultados: Al comparar los grupos (100 casos y 194 controles) se encontraron diferencias estadísticamente significativas en la TA sistólica y diastólica, semanas de gestación e IMC. En la edad y antecedente de preeclampsia no hubo diferencias entre los grupos. Las frecuencias alélicas fueron T=0.65, G=0.35 (controles), T=0.72 y G=0.28 (casos). Las frecuencias genotípicas fueron T/T=0.42, T/G=0.47, G/G=0.11 (controles), T/T=0.53, T/G=0.39, G/G=0.08 (casos). Aunque estadísticamente no se encontró asociación entre el polimorfismo rs2228001 y la preeclampsia al estimar la OR en los modelos de herencia codomiante, dominante y recesivo, el modelo dominante muestra una tendencia hacia la probabilidad de que este polimorfismo sea un factor protector (OR=0.64, IC95=0.39-1.04).

Conclusiones: Aunque las evidencias sugieren que el polimorfismo rs2228001 del gen *XPC* no se relaciona con la preeclampsia, es necesario realizar nuevos estudios con tamaño de muestra mayores en población mexicana para confirmar nuestros resultados.

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Por la presentación del trabajo:

Genotipificación del polimorfismo rs2228001 del gen *XPC* en mujeres con preeclampsia y embarazo normoevolutivo

durante XLIX Congreso Nacional de Ciencias Farmacéuticas y VII Congreso Internacional de Ciencias Farmacéuticas Huatulco - 4 al 7 de septiembre 2016

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TRABAJO LIBRE - BDM00026

Caracterización del polimorfismo *MTHFR* C677T en pacientes con enfermedad de Parkinson

Sandoval Carrillo Ada[1], *Medina Simental Rosa Arlette* [1], *Ramírez Sosa Lino Enrique*[1], *Méndez Hernández Edna Madai*[1], *Salas Pacheco José Manuel*[1], *Miranda Morales Ernesto Gerardo*[1], *Arias Carrion Oscar*[2]

[1]INSTITUTO DE INVESTIGACIÓN CIENTÍFICA DE LA UJED
[2]HOSPITAL GENERAL MANUEL GEA GONZALEZ

Introducción: La enfermedad de Parkinson (EP) es uno de los trastornos neurodegenerativos más frecuentes. Clínicamente se caracteriza por bradicinesia, rigidez y temblor. Los defectos en la función motora se deben a una degeneración progresiva de las neuronas dopaminérgicas de la sustancia nigra pars compacta. Las bases genéticas y bioquímicas asociadas a la EP en población Mexicana han sido poco estudiadas. Diversos estudios han arrojado resultados controversiales al evaluar la posible asociación entre el polimorfismo *MTHFR* C677T y la EP. En el 2013 Wu y cols. encontraron una asociación entre este polimorfismo y la EP en población caucásica y asiática Sin embargo, en el 2013 Liao y cols. encontraron que no existe una asociación entre este polimorfismo y la EP en población China.

Objetivo: Determinar si existe una asociación entre el polimorfismo *MTHFR* C677T y la EP en población Mexicana.

Metodología: Estudio prospectivo de casos (n=100) y controles (n=100). Se tomaron muestras de sangre periférica a partir de la cual se extrajo ADN utilizando el sistema QIAamp DNA Blood Mini Kit. La genotipificación se realizó utilizando sondas Taqman en un equipo STEP ONE de 48 pozos.

Resultados: Al analizar los grupos encontramos una edad media de 69.4 años. 56% de nuestra población fueron mujeres. En el grupo de casos se encontró que el 9% tiene antecedentes familiares de EP, 16% discinesias y 35% trastornos del sueño. Las frecuencias alélicas fueron T=0.56 y C=0.44 para los controles y T=0.55 y C=0.45 para los casos. Las frecuencias genotípicas fueron T/T=0.29, T/C=0.53 y C/C=0.18 para los casos y T/T=0.33, T/C=0.43 y C/C=0.24 para los controles. No hubo diferencias estadísticamente significativas entre los grupos. Al estimar el riesgo (OR) de EP conferido por el polimorfismo en los modelos de herencia codominante, dominante y recesivo, no se encontró ninguna significancia estadística. Al estimar la OR estratificando por genero tampoco se encontraron significancias estadísticas.

Conclusiones: Los resultados de éste análisis muestran que el polimorfismo *MTHFR* C677T no es un factor de riesgo para la Enfermedad de Parkinson en población mexicana, resultado semejante al reportado en población China.

TRABAJO LIBRE - BDM00027

Polimorfismos en genes codificantes de transportadores de urato asociados con deterioro cognitivo en adulto mayor

Carrillo Leyva Pedro[1], *Méndez Hernández Edna Madai*[2], *Miranda Morales Ernesto Gerardo*[2], *Fernández Chávez Ana Gabriela*[1], *Castellanos Juárez Francisco Xavier*[2], *Basio Salazar Carolina*[2]

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Introducción: Diversos estudios han establecido asociación entre niveles reducidos de ácido úrico (AU) y un incremento en el riesgo de desarrollar deterioro cognitivo. Los principales reguladores del AU son los transportadores GLUT-9 (SLC2A9) y URAT-1 (SLC22A12). La presencia de variantes génicas en estos, influyen sobre los niveles de AU al modular su excreción por vía renal, por lo que pudieran representar factores de riesgo para padecimientos neurodegenerativos.

Objetivo: Establecer si existe asociación entre polimorfismos en genes codificantes de transportadores de urato asociados con la presencia y severidad de deterioro cognitivo en el adulto mayor.

Metodología: Estudio de casos y controles. Se estudiaron adultos mayores evaluados con la escala Mini-mental test de Folstein (MMSE) para identificar la presencia de deterioro o normalidad cognitiva. Se evaluaron niveles séricos y urinarios de AU. La genotipificación se realizó por qPCR.

Resultados: Se incluyeron 121 sujetos (31 casos, 90 controles). Al comparar las variables de estudio se observó edad (74.8±7.0 vs 72.7±6.8, p=0.201), IMC (25.9 (23.7-27.5) vs 28.5 (25.1-31.2), p=0.104), MMSE (19.0 (8-21) vs 29.0 (27.7-30.0), p=0.000), AU (4.1±1.7 vs 4.8±1.6, p=0.056), FEAU (31 (21.0-345.8 vs 23.8 (7.0-43.9), p=0.313), frecuencia de hipouricemia (0 (0) vs 5 (17.2), p 0.000), entre casos y controles respectivamente. En el análisis por género únicamente se observaron diferencias en las variables AU (3.4±1.7 vs 4.7±1.7, p=0.008) y MMSE (18.0 (5.0-20.0) vs 29.0 (26.7-30.0), p=0.000) al comparar entre casos y controles en las mujeres. En los hombres solamente se encontró diferencia en la variable MMSE (19 (8-21) vs 29.0 (28.0-30.0), p=0.000). No se observaron diferencias al comparar las frecuencias alélicas (p 0.712) y genotípicas (p 0.936) de la variante rs733175. Al comparar los niveles de AU entre los diferentes genotipos de esta variante observamos (4.65±1.7, 4.8±1.7 y 4.2±1.7, p=0.407) en homocigotos silvestres, heterocigotos y homocigotos mutados respectivamente.

Conclusiones: Se observaron niveles reducidos de AU en hombres con deterioro cognitivo, este comportamiento no se observó en las mujeres. El AU es un poderoso captador de radicales libres al cual se le atribuyen propiedades neuroprotectoras. Este pudiera ser el mecanismo por el cual pudiera asociarse a la funcionalidad cognitiva.

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TRABAJO LIBRE - BDM00028

Polimorfismos en los genes IL-18, IL-12, TLR4 y TLR9 y depresión en mujeres embarazadas

Sandoval Carrillo Ada[1], Salas Pacheco Sergio Manuel[1], Barragán Ávila Efrén[1], Alvarado Esquivel Cosme[2], Hernández Tinoco Jesus[1], Antuna Salcido Irasema[1], Salas Pacheco José Manuel[1]

[1]INSTITUTO DE INVESTIGACIÓN CIENTÍFICA DE LA UJED
[2]FACULTAD DE MEDICINA Y NUTRICIÓN DE LA UJED

Introducción: Alrededor del 10% de las mujeres embarazadas y el 13 de las mujeres que acaban de dar a luz experimentan un desorden mental, principalmente depresión. Actualmente existe evidencia que demuestra que la depresión está asociada con una respuesta inflamatoria crónica de bajo grado, activación inmune mediada por células y activación compensatoria del sistema reflejo anti-inflamatorio. Por tal motivo, el estudio de variantes en genes que participan en estos procesos es de interés para diversos grupos de investigación, incluyendo el nuestro.

Objetivo: Determinar si existe asociación entre los polimorfismos IL-18 -137C/G, IL-12 1188A/C, TLR4 896A/G y TLR2 1350T/C y la depresión en mujeres embarazadas.

Metodología: Estudio de casos (embarazadas con depresión, n=153) y controles (embarazadas sin depresión, n=177). La depresión se evaluó mediante la Escala de Depresión de Post-Parto de Edinburgh. Se tomaron muestras de sangre periférica a partir de la cual se extrajo ADN utilizando el sistema QIAamp DNA Blood Mini Kit. La genotipificación se realizó por PCR en tiempo real utilizando sondas Taqman en un equipo STEP ONE de 48 pozos.

Resultados: Al analizar las frecuencias alélicas y genotípicas de los polimorfismos de estudio, solo encontramos diferencias estadísticamente significativa entre los grupos en el polimorfismo IL-12 1188A/C. Las frecuencias alélicas fueron A=0.64 y C=0.36 para los casos y A=0.55 y C=0.45 para los controles (p= 0.021). Las frecuencias genotípicas fueron A/A=0.39, A/C=0.51 y C/C=0.10 para los casos y A/A=0.31, A/C=0.49 y C/C=0.20 para los controles (p=0.038). De la misma forma, al estimar el riesgo mediante la OR, el genotipo C/C mostró ser un factor protector (OR=0.42, IC95=0.20-0.85). El alelo A se ha asociado con un incremento del 50% de transcritos por lo que nuestros resultados sugieren que aquellos individuos que presentan el genotipo C/C tendrían menores niveles de la interleucina 12 proinflamatoria. Como consecuencia, una respuesta inflamatoria disminuida estaría actuando como un factor protector para el desarrollo de la depresión.

Conclusiones: El genotipo C/C del polimorfismo A/C 1188 del gen IL-12 es un factor protector para la depresión en mujeres embarazadas.

TRABAJO LIBRE - BDM00030

Caracterización de los polimorfismos -857C/T y -238G/A del gen *TNF*α en mujeres embarazadas con depresión

Sandoval Carrillo Ada[1], Alvarado Esquivel Cosme[2], Sánchez Anguiano Luís Francisco[1], Hernández Tinoco Jesus[1], Salas Pacheco Sergio Manuel[1], Barragán Ávila Efrén[1], Salas Pacheco José Manuel[1]

[1]INSTITUTO DE INVESTIGACIÓN CIENTÍFICA DE LA UJED
[2]FACULTAD DE MEDICINA Y NUTRICIÓN DE LA UJED

Introducción: La depresión es común, especialmente en mujeres con edad fértil, de las cuales entre un 8 a un 12% padecen de depresión. Se ha documentado que diferentes tipos de depresión mantienen una relación cercana con la respuesta inmunitaria, específicamente la inflamación y todas sus moléculas involucradas. *TNFα* tiene propiedades reguladoras de inflamación, mismas que juegan un papel crucial en la inmunidad innata y adaptativa. Polimorfismos en el gen *TNFα* se han asociado con variabilidad en su funcionalidad.

Objetivo: Determinar el papel de los polimorfismos -857C/T y -238G/A del gen *TNFα* en mujeres embarazadas con depresión.

Metodología: Estudio de casos (embarazadas con depresión, n=153) y controles (embarazadas sin depresión, n=177). La depresión se evaluó mediante la Escala de Depresión de Post-Parto de Edinburgh. De sangre periférica se extrajo ADN (QIAamp DNA Blood Mini Kit). Se genotipificó por PCR en tiempo real en un equipo STEP ONE de 48 pozos.

Resultados: La media de edad fue de 23.49 y 23.58 años (casos y controles, respectivamente). Las frecuencias alélicas y genotípicas del polimorfismo -857C/T fueron C=0.77, T=0.23, C/C=0.59, C/T=0.37, T/T=0.04 y C=0.84, T=0.16, C/C=0.72, C/T=0.25, T/T=0.03 para los casos y controles, respectivamente. Al comparar las frecuencias entre los grupos encontramos diferencias estadísticamente significativas en las alélicas (p=0.30) y genotípicas (p=0.047). Para el polimorfismo -238G/A las frecuencias alélicas y genotípicas fueron G=0.97, A=0.03, G/G=0.95, G/A=0.05, A/A=0 y G=0.92, A=0.08, G/G=0.84, G/A=0.16, A/A=0.2 para los casos y controles, respectivamente. Al comparar las frecuencias entre los grupos encontramos diferencias estadísticamente significativas en las alélicas (p=0.0019). Posteriormente se calcularon las OR encontrándose que el genotipo C/T del polimorfismo -857C/T es un factor de riesgo (OR=1.78, IC95=1.09-2.89), mientras que el genotipo G/A es un factor protector (OR=0.33, IC95=0.14-0.75). *TNFα* es un mediador de la respuesta inflamatoria en ambas direcciones, tanto proinflamatoria como antiinflamatoria, lo que quizá explique nuestros resultados. Estudios de funcionalidad para ambos polimorfismos son necesarios para determinar el papel que juega cada uno de ellos.

Conclusiones: El polimorfismo -857C/T del gen *TNFα* es un factor de riesgo y el polimorfismo -238G/A del gen *TNFα* es un factor protector, ambos, para la depresión en mujeres embarazadas.

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TRABAJO LIBRE - BDM00030

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Otorga la presente

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a: **Ada Agustina Sandoval Carrillo, Cosme Alvarado Esquivel, Luis Francisco Sánchez Anguiano, Irasema Elizabeth Antuna Salcido, Sergio Manuel Salas Pacheco, Lilia Martina Vélez Vélez, Francisco Xavier Castellanos**

Por su participación como **Ponente en la Modalidad de Póster** dentro de la

II Jornada Nacional de Investigación en Salud Durango 2018

Con el tema:

Epidemiología de la Infección por Leptospira en la Población General de la Ciudad de Durango, México

los días 18, 19 y 20 de octubre del 2018, en el Centro Cultural y de Convenciones Bicentenario, Durango, Dgo.

Victoria de Durango, Dgo. octubre de 2018

Dr. José Rosas Aispuro Torres
GOBERNADOR DEL ESTADO DE DURANGO

Dr. Sergio González Romero
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Por la presentación del trabajo ***"CARACTERIZACIÓN DE LOS PATRONES DE EXPRESIÓN DE LOS GENES CLOCK Y VARIABLES POLISOMNOGRÁFICAS EN PACIENTES CON ENFERMEDAD DE PARKINSON"****, realizado en las Jornadas Académicas "La Investigación Científica, Compromiso y Pertinencia Social", en el marco conmemorativo del XLVIII Aniversario del IIC y II Encuentro de Investigación de la DES - Ciencias de la Salud de la UJED.*

Atentamente

" Por mi raza hablará el espíritu "

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Rubella Immune Status in Pregnant Women in a Northern Mexican City

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Abstract

Background: The seroepidemiology of rubella virus infection in pregnant women in northern Mexico is largely unknown. We sought to determine the seroprevalence of rubella virus infection in pregnant women in the northern Mexican city of Durango, Mexico. Seroprevalence association with the socio-demographic, clinical and behavioral characteristics of the pregnant women was also investigated.

Methods: Through a cross-sectional study, we determined the seroprevalence of IgG and IgM anti-rubella virus in 279 pregnant women (mean age 29.17 ± 5.96 years; range 15 - 43 years) attending in a clinic of family medicine using enzyme-linked fluorescent assays. A questionnaire was used to obtain the socio-demographic, clinical and behavioral characteristics of the pregnant women. The association of rubella seropositivity and characteristics of the women was assessed by bivariate and multivariate analyses.

Results: Anti-rubella IgG antibodies (≥ 15 IU/mL) were found in 271 (97.1%) of the 279 pregnant women examined. None of the 279 pregnant women were positive for anti-rubella IgM antibodies. Multivariate analysis of socio-demographic, clinical and behavioral variables showed that seroreactivity to rubella virus was positively associated with national trips (OR = 7.39; 95% CI: 1.41 - 38.78; P = 0.01), and negatively associated with age (OR = 0.26; 95% CI: 0.06 - 0.99; P = 0.04).

Conclusions: Rate of rubella immunity in pregnant women in the northern Mexican city of Durango is high. However, nearly 3% of pregnant women are susceptible to rubella in our setting. Risk factors associated with rubella seropositivity found in this study may be useful for optimal design of preventive measures against rubella and its sequelae.

Keywords: Rubella; Pregnant women; Epidemiology; Seroprevalence; Cross-sectional study; Mexico

Introduction

Rubella virus is a single-stranded ribonucleic acid virus of the Togaviridae family [1, 2], and is a sole member of the genus Rubivirus [3]. Infection with rubella virus occurs by inhalation of contaminated droplets [1], and can be vertically transmitted to fetuses during maternal infection leading to congenital infection [4]. Rubella virus is an important pathogen worldwide [5]. Infection with rubella virus causes a febrile rash illness in children and adults [6]. In addition, infection with rubella virus in adults may cause severe inflammation and pain in the joints [1]. However, infection with rubella virus during the first trimester of pregnancy can lead to prematurity, low birth weight [7], miscarriage, stillbirth [6], and congenital rubella syndrome [6, 8]. This syndrome is characterized by fetal anomalies including mental retardation [9], heart defects, cataracts [8], blindness, deafness [9], and hepatomegaly and jaundice [10]. There is not currently antiviral treatment for rubella [1]. An effective and sure vaccine against rubella is available [1, 5]. However, rubella outbreaks in Japan and other countries have been reported recently [2, 5, 11].

The seroepidemiology of rubella virus infection in Mexican populations has been scantily studied. An 87% seroprevalence of anti-rubella antibodies in puerperal women from Delicias City in the northern Mexican city of Chihuahua was reported [12], whereas a 92.6% seroprevalence of rubella virus infection in pregnant women in two zones of the valley of Mexico was found [13]. In a study in Leon, Guanajuato, Mexico, researchers found a 71% seroprevalence of rubella in 176 women at reproductive age [14]. To the best of our knowledge, there is not any study on the seroepidemiology of rubella virus infection in pregnant women in northern Mexico. Therefore, this study was aimed to determine the seroprevalence of rubella virus infection in pregnant women in the northern Mexican city of Durango, Mexico. Furthermore, rubella seroprevalence

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association with the socio-demographic, clinical and behavioral characteristics of the pregnant women was also assessed.

Materials and Methods

Study design and population

We performed a cross-sectional study using stored serum samples from a previous survey of cytomegalovirus infection in pregnant women in Durango City, Mexico [15]. Samples were originally obtained to determine the seroprevalence of anti-cytomegalovirus antibodies in pregnant women attending a public primary health care center (Clinic of Family Medicine, Institute of Security and Social Services of State Workers) in Durango City, Mexico. Serum samples were obtained from April to November 2013. Inclusion criteria for enrollment of the participants were: 1) pregnant women attending prenatal care; 2) aged 15 years and older; 3) residence in Durango City; and 4) who voluntarily accepted to participate in the survey.

Socio-demographic, clinical and behavioral characteristics of participants

We obtained the socio-demographic, clinical and behavioral characteristics from the participants with the aid of a questionnaire. Socio-demographic items included age, birthplace, residence, educational level, occupation and socio-economic status. Clinical items included health status, history of lymphadenopathy, frequent headaches; impairments of memory, vision and hearing; and history of blood transfusions. In addition, the obstetric history (month of pregnancy, number of pregnancies, deliveries, cesarean sections and miscarriages) from each participant was recorded. Behavioral items included foreign traveling, alcohol consumption, tobacco use, and washing hands before eating.

Laboratory tests

Sera of the participants were kept frozen until analyzed. Sera were examined for anti-rubella IgG antibodies by a commercially available enzyme-linked fluorescent assay (ELFA) "VIDAS RUB IgG II" kit (bioMerieux SA, Marcy-l'Etoile, France) and for anti-rubella IgM antibodies by a commercially ELFA "VIDAS RUB IgM" kit (bioMerieux SA, Marcy-l'Etoile, France). Anti-rubella IgG antibody levels of $\geq$ 15 IU/mL were considered as a cut-off for seropositivity. This titer suggests protection against rubella [16, 17]. All tests were performed according to the manufacturer's instructions.

Ethical aspects

This study was performed using stored serum samples from a previous survey. In such previous study, the purpose and procedures of the study were explained to all participants, and a written informed consent was obtained from all of them and from the next of kin of minor participants. The ethical committee of the Institute of Security and Social Services of State Workers in Durango City, Mexico approved this study.

Statistical analysis

Results were analyzed with the aid of the Epi Info version 7 and SPSS version 15.0 software. For calculation of the sample size, we used a value of 15,000 as a population size from which the sample was selected, a reference seroprevalence of 87.0% [12] as expected frequency of the factor under study, 5.0% of confidence limits, a design effect of 1.0, one cluster, and a confidence level of 95%. The result of the calculation was 172 subjects. We evaluated the association between the characteristics of the women and rubella seropositivity by using bivariate and multivariate analyses. For comparison of the frequencies among groups, the Pearson's Chi-square and the Fisher exact test (when values were less than 5) were used. As a strategy to include variables in the multivariate analysis, we selected only variables with a P value equal to or less than 0.05 obtained in the bivariate analysis. We calculated the odds ratios (ORs) and 95% confidence intervals (CIs) by multivariate analysis using the Enter method. Statistical significance was set at a P value < 0.05.

Results

We enrolled a total of 279 pregnant women. Their mean age was 29.17 ± 5.96 years (range 15 - 43 years). Table 1 shows the general socio-demographic characteristics of the pregnant women studied. Anti-rubella IgG antibodies were found in 271 (97.1%) of the 279 pregnant women examined. None of the 279 pregnant women were positive for anti-rubella IgM antibodies. Of the socio-demographic characteristics of the pregnant women, the variables including age group and socioeconomic status were associated with anti-rubella IgG antibodies by bivariate analysis, whereas the variables including birthplace, residence, educational level, and occupation did not associate with anti-rubella IgG antibodies.

With respect to clinical characteristics, rubella seroprevalence was similar in ill and healthy pregnant women. Table 2 shows a correlation of rubella seroprevalence and clinical characteristics of pregnant women. Rubella seroprevalence was significantly (P = 0.02) higher in pregnant women with frequent headaches, whereas women with history of deliveries had a higher (borderline significance: P = 0.05) rubella seroprevalence than women without this history. Other clinical characteristics of women including history of lymphadenopathy, impairments of memory, vision and hearing; history of blood transfusions, month of pregnancy, number of pregnancies, deliveries, cesarean sections and miscarriages did not show an association with rubella seroprevalence by bivariate analysis.

Concerning behavioral characteristics of women, the variable "national trips" showed a borderline (P = 0.05) association with rubella seroprevalence. Other behavioral characteristics

Table 1. Socio-Demographic Characteristics of Pregnant Women and Seroprevalence of Rubella IgG Antibodies (≥ 15 IU/mL)

Characteristic	No. of women tested[a]	Rubella seroprevalence		P value
		No.	%	
Age groups (years)				
15 - 24	61	60	98.4	0.01
25 - 34	159	157	98.7	
35 - 43	59	54	91.5	
Birth place				
Durango State	259	252	97.3	1.00
Other Mexican State	16	16	100.0	
Residence place				
Durango State	278	270	97.1	1.00
Other Mexican State	1	1	100.0	
Residence area				
Urban	264	257	97.3	0.42
Suburban	4	4	100.0	
Rural	11	10	90.9	
Educational level				
Up to 6 years	1	1	100.0	0.30
7 - 12 years	102	97	95.1	
13 or more years	176	173	98.3	
Occupation				
Unemployed[b]	81	78	96.3	0.69
Employed[c]	198	193	97.5	
Socioeconomic level				
Low	15	13	86.7	0.02
Medium	257	252	98.1	
High	3	3	100.0	

[a]Sums may not add up to 279 because of some missing values. [b]Unemployed: none occupation, student or housewife. [c]Employed: employee, professional, business, or other.

including traveling abroad, alcohol consumption, tobacco use, and washing hands before eating did not show an association with rubella seroprevalence.

Multivariate analysis of socio-demographic, clinical and behavioral variables with P values ≤ 0.05 by bivariate analysis including age, socioeconomic status, frequent headache, number of deliveries, and national trips showed that seroreactivity to rubella was positively associated only with national trips (OR = 7.39; 95% CI: 1.41 - 38.78; P = 0.01), and negatively associated only with age (OR = 0.26; 95% CI: 0.06 - 0.99; P = 0.04).

Discussion

Very little is known about the serological status against rubella virus in pregnant women in Mexico. Therefore, this study aimed to determine the seroprevalence of IgG and IgM antibodies against rubella virus in pregnant women in the northern Mexican city of Durango. Results indicate that 97.1% of the pregnant women studied had protective (≥ 15 IU/mL) antibodies against rubella virus infection. In Mexico, vaccination against rubella started in 1998 [18]. Although the majority of pregnant women tested had protective antibodies, nearly 3% of women were susceptible to rubella. This figure seems low but considering that there are nearly 40,000 births a year in Durango State (http://cuentame.inegi.org.mx/monografias/informacion/dur/poblacion/dinamica.aspx?tema=me&e=10), thus there are about 1,200 pregnant women susceptible to rubella virus just in this Mexican state. Concerning studies in Mexico, the seroprevalence of rubella found in the present study is higher than the 87% seroprevalence of rubella in early puerperium women in the northern Mexican city of Delicias, Chihuahua [12], the 92.6% seroprevalence of rubella in pregnant women from Iztapalapa and Nezahualcoyotl areas in the valley of Mexico [13], and the 71% seroprevalence in women

Table 2. Bivariate Analysis of Clinical Data and Seropositivity to Rubella Virus in Pregnant Women in Durango City, Mexico

Characteristic	No. of women tested[a]	Rubella prevalence		P value
		No.	%	
Clinical status				
Healthy	267	260	97.4	0.27
Ill	11	10	90.9	
Lymphadenopathy ever				
Yes	42	41	97.6	1.00
No	237	230	97.0	
Headache frequently				
Yes	112	112	100.0	0.02
No	167	159	95.2	
Memory impairment				
Yes	63	63	100.0	0.20
No	216	208	96.3	
Hearing impairment				
Yes	20	20	100.0	1.00
No	259	251	96.9	
Visual impairment				
Yes	79	76	96.2	0.69
No	199	194	97.5	
Blood transfusion				
Yes	13	12	92.3	0.32
No	265	258	97.4	
Pregnancies				
One	89	89	100.0	0.11
Two	97	93	95.9	
Three	50	49	98.0	
Four	31	28	90.3	
Five	9	9	100.0	
More than 5	2	2	100.0	
Deliveries				
Zero	157	153	97.5	0.05
One	65	63	96.9	
Two	41	41	100.0	
Three	11	9	81.8	
Four	3	3	100.0	
More than 4	1	1	100.0	
Cesarean sections				
Zero	195	189	96.9	0.72
One	62	61	98.4	
Two	21	20	95.2	
Miscarriages				
Zero	223	217	97.3	0.88
One	46	44	95.7	
Two	8	8	100.0	
Three	1	1	100.0	
Month of pregnancy				
1 - 3	100.0	98	98.0	0.1
4 - 6	118	116	98.3	
7 - 9	56	52	92.9	

[a]Sums may not add up to 279 because of some missing values.

of reproductive age in Leon, Guanajuato [14]. However, this comparison should be taken with care since these studies were performed in different years and laboratory tests used were different from the tests we used. Previous seroprevalence studies in Mexico were performed from 1993 to 2004. In those years, the coverage of rubella vaccination was lower than the one in the recent years. We used ELFA to detect IgG antibodies against rubella virus, whereas in the previous studies, the hemagglutination inhibition method [13, 14] was used. In addition, we studied pregnant women in the urban city of Durango, whereas rural and urban women were enrolled in the study in Delicias, Chihuahua [12]. In an international context, the seroprevalence of rubella in pregnant women in Durango is higher than the 93.1% seroprevalence of rubella found in pregnant women seen in a tertiary hospital in Zaria, Nigeria [19], and 87.5% seroprevalence in pregnant women in Osogbo, Nigeria [20] using enzyme-linked immunosorbent assays. Similarly, our prevalence is higher than the 85.8% seroprevalence reported in pregnant women in southern Italy using a microparticle enzyme immunoassay [21]. The rubella seroprevalence found in our study is comparable with the 95.1% seroprevalence of rubella reported in pregnant women in Sudan [22], the 93.3% seroprevalence in pregnant women in Portugal [23], the 94.4% seroprevalence in pregnant women in Oslo, Norway [24], and 95.4% seroprevalence in women of childbearing age in Venezuelan Yupka indigenous communities [25].

We searched for factors associated with rubella seroprevalence. We found that seroreactivity to rubella was positively associated with national trips and negatively associated with age. International travel has been linked to rubella importation in the USA [26]. We did not find an association of international travel with rubella seropositivity. However, it is possible that rubella exposure occurs also by national trips as results of the present study suggests. Therefore, traveling to high endemic rubella regions should be avoided by pregnant women. In the present study, seroprevalence decreases with age. This fact might reflect the higher coverage of rubella vaccination in young women.

This study has limitations including a small sample size, and enrollment of women in only one clinic of family medicine. Further studies with larger sample sizes and in several clinics to determine the seroprevalence of rubella in Mexican communities should be conducted.

Conclusions

Rate of rubella immunity in pregnant women in the northern Mexican city of Durango is high. However, nearly 3% of pregnant women are susceptible to rubella in our setting. Risk factors associated with rubella seropositivity found in this study may be useful for optimal design of preventive measures against rubella and its sequelae.

Conflicts of Interest

The authors declare that no conflicts of interest exist.

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High Prevalence of *Toxoplasma gondii* Infection in Miners: A Case-Control Study in Rural Durango, Mexico

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Abstract

Background: Very little is known about the seroepidemiology of infection with the parasite *Toxoplasma gondii (T. gondii)* in miners. We determine the association of *T. gondii* infection and the occupation of miner, and the association of seropositivity for *T. gondii* with the socio-demographic, clinical, work and behavioral characteristics of the miners.

Methods: Through a case-control study, 125 miners working in Durango State, Mexico and 250 age- and gender-matched non-miner subjects were examined for the presence of anti-*T. gondii* IgG and IgM antibodies using enzyme-linked immunoassays. In addition, the presence of *T. gondii* DNA in miners was determined using polymerase chain reaction. Bivariate and multivariate analyses were used to determine the association of socio-demographic, work, clinical and behavioral characteristics of miners with *T. gondii* infection.

Results: Anti-*T. gondii* IgG antibodies were detected in 75 (60.0%) of 125 miners and in 55 (22.0%) of 250 controls (odds ratio (OR) = 5.31; 95% confidence interval (CI): 3.33 - 8.47; P < 0.001). Among IgG seropositive subjects, the frequency of anti-*T. gondii* IgM antibodies was significantly higher in miners (39/75, 52%) than in controls (8/55, 14.5%) (P < 0.001). All *T. gondii* seropositive miners referred themselves as healthy. Multivariate analysis of socio-demographic, housing, and behavioral characteristics of miners showed that *T. gondii* seropositivity was positively associated with being born in Durango State (OR = 3.44; 95% CI: 1.09 - 10.7; P = 0.03), consumption of boar meat (OR = 5.53; 95% CI: 1.49 - 20.3; P = 0.01), living in an overcrowded home (OR = 5.83; 95% CI: 1.49 - 22.8; P = 0.01), and was negatively associated with cleaning cat excrement (OR = 0.33; 95% CI: 0.11 - 0.90; P = 0.03) and consuming goat meat (OR = 0.16; 95% CI: 0.03 - 0.76; P = 0.02).

Conclusions: Surprisingly, our results indicate that miners represent a risk group for *T. gondii* infection. This is the first age- and gender-matched case-control study on the association of *T. gondii* infection and the occupation of miner. Further studies to identify the exact cause of high seropositivity in miners in rural Durango are needed.

Keywords: *Toxoplasma gondii*; Infection; Seroprevalence; Miners; Case-control study; Epidemiology; Mexico

Introduction

Infections with the parasite *Toxoplasma gondii (T. gondii)* are common in humans around the world [1]. These infections may lead to toxoplasmosis characterized by lymph node enlargement, chorioretinitis, or neuropsychiatric manifestations [2, 3]. Immunocompromised subjects infected with *T. gondii* may develop severe to life-threatening symptoms, most often toxoplasmic encephalitis [4]. In addition, a primary infection with *T. gondii* in pregnant women may lead to fetal infection and congenital toxoplasmosis [2, 5]. Infection with *T. gondii* is typically acquired by ingestion of raw or undercooked meat containing viable tissue cysts [6], or by ingestion of food or water contaminated with oocysts shed by cats [7]. Other routes of *T. gondii* infection are thought to be rare including organ transplantation [8] and blood transfusion [9].

The epidemiology of *T. gondii* infection in miners has been scantly studied, and we are not aware of any study of this infection in miners in Mexico. The epidemiological link between miners and *T. gondii* may be the close contact with soil and water that could be contaminated with oocyst shed by cats or other felids. In addition, miners work in rural areas where hunting of wild animals is common, and the risk for acquiring infection by eating raw or undercooked meat from *T. gondii*-infected animals is high. The seroprevalence of infection with *T. gondii* and its

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association with risk factors for infection in miners in Mexico are largely unknown. Therefore, we sought to determine the seroprevalence of *T. gondii* infection in miners in a municipality in rural Durango, Mexico, and to determine the association of seropositivity for *T. gondii* with the socio-demographic, clinical, work and behavioral characteristics of the miners.

Materials and Methods

Study design and population groups studied

We performed a case-control seroprevalence study of 125 miners (cases) and 250 age- and gender-matched non-miner subjects (controls). Cases and controls were examined for the presence of anti-*T. gondii* IgG and IgM antibodies. Miners were enrolled from December 2015 to August 2016 in a mine located in the San Dimas Municipality, in the northern Mexican state of Durango. Inclusion criteria for the miners were as follows: 1) working in the mine for at least 3 months, 2) 18 years and older, and 3) willing to participate in the study. All cases included in the study were males and had been working from 3 months to 47 years (mean: 11.4 ± 9.5 years) as miners. They were 20 - 87 (mean: 43.8 ± 14.6) years old. Controls were subjects randomly selected from the general population in rural Durango [10]. Controls were matched with cases by gender and age. Controls were males aged 20 - 87 (mean: 43.85 ± 14.5) years old and their age did not differ from that in cases (P = 0.99).

Socio-demographic, housing, clinical, work, and behavioral data of miners

Socio-demographic, clinical, work, and behavioral characteristics of the miners were recorded with the aid of a questionnaire. Socio-demographic items included age, gender, birthplace, residence, and socioeconomic level. Housing conditions items included availability of potable water, form of elimination of excretes, years of education of the head of the family, type of flooring at home, and crowding. Assessment of crowding was performed by dividing the number of people by the number of bedrooms in a house. "Semi-crowed" was considered when 1.6 - 3.5 people were living in a single bedroom. "Overcrowded" was considered when 3.6 or more people were living in a single room. The socioeconomic level of participants was self-reported. A "low socioeconomic status" was considered when a participant lived in poverty, whereas a "medium socioeconomic status" was considered when a participant did not live in poverty or wealth but in an intermediate status.

Clinical data included presence of any illness, history of lymphadenopathy, surgery, transplant, or blood transfusion, impairments in memory, reflexes, hearing and vision, frequent abdominal pain or headache, and dizziness. We recorded the duration of the activity as a miner for each participant. Behavioral items were contact with cats or other animals, cleaning cat feces, foreign traveling, type of meat consumed, frequency of meat consumption, eating raw or undercooked meat, animal brains, beef liver, and dried or cured meat, untreated water or unpasteurized milk, and unwashed raw vegetables or fruits. In addition, behavioral items included washing hands before eating, frequency of eating out of home (in restaurants or fast food outlets), alcoholism, tobacco smoking, and soil contact.

Detection of *T. gondii* IgG and IgM antibodies

Serum samples of miners were analyzed for anti-*T. gondii* IgG antibodies with the commercially available enzyme immunoassay kit "*Toxoplasma* IgG" (Diagnostic Automation Inc., Woodland Hills, CA, USA). Levels of anti-*T. gondii* IgG antibody were expressed as International Units (IU)/mL, and a result ≥ 8 IU/mL was considered positive. Serum samples with anti-*T. gondii* IgG antibodies were further tested for anti-*T. gondii* IgM antibodies by the commercially available enzyme immunoassay "*Toxoplasma* IgM" kit (Diagnostic Automation Inc.). All assays were performed according to the manufacturer's instructions. Positive and negative controls included in the kits were included in each run.

Extraction of DNA and detection of *T. gondii* DNA

Miners with *Toxoplasma*-specific IgG antibodies by EIA were further examined for *T. gondii* DNA by nested-polymerase chain reaction (PCR). Extraction of DNA from whole blood was performed following a protocol described by Iranpour and Esmailizadeh (http://www.protocol-online.org/prot/Protocols/Rapid-Extraction-of-High-Quality-DNA-from-Whole-Blood-Stored-at-4-C-for-Long-Period-4175.html). PCR amplification was performed with primers directed against the B1 gene of *T. gondii* and following the protocol described by Roth et al [11]. Amplification products were analyzed with 2% agarose gel electrophoresis, stained with ethidium bromide, and visualized by ultraviolet transillumination.

Statistical analysis

Results were analyzed with the aid of the software Microsoft Excel 2010, Epi Info version 7 (Centers for Disease Control and Prevention: http://wwwn.cdc.gov/epiinfo/) and SPSS version 15.0 (SPSS Inc. Chicago, IL). For calculation of the sample size, we used a 95% confidence level, a power of 80%, a 1:2 proportion of cases and controls, a reference seroprevalence of 23.8% [10] as the expected frequency of exposure in controls, and an odds ratio (OR) of 2. The result of the sample size calculation was 115 cases and 229 controls. We compared the age of cases and controls using the Student's *t*-test. The Pearson's Chi-square test and the Fisher exact test (when values were small) were used to determine the association of *T. gondii* seropositivity with the characteristics of miners. As a strategy for multivariate analysis, only characteristics of miners with a P value ≤ 0.10 obtained in the bivariate analysis were included in the analysis. OR and 95% confidence interval (CI) were calculated using logistic regression analysis with the Enter method. A P value < 0.05 was considered as statistically

Table 1. Socio-Demographic and Housing Characteristics of Miners and Prevalence of *T. gondii* Infection

Characteristics	Total subjects	Prevalence of *T. gondii* infection		P value
		No.	%	
Age groups (years)				
30 or less	26	16	61.5	0.73
31 - 50	59	37	62.7	
> 50	40	22	55.0	
Birth place				
Durango State	98	64	65.3	0.02
Other Mexican State	27	11	40.7	
Educational level				
No education	16	9	56.3	0.72
1 - 6 years	68	42	61.8	
7 - 12 years	36	20	55.6	
> 12 years	5	4	80.0	
Socio-economic level				
Low	61	42	68.9	0.04
Medium	64	33	51.6	
Flooring at home				
Ceramic or wood	5	3	60.0	0.87
Concrete	92	54	58.7	
Soil	28	18	64.3	
Availability of potable water				
In home	2	1	50.0	0.34
In land	3	3	100.0	
In street	120	71	59.2	
Toilet facilities				
Sewage pipes	5	3	60.0	1.00
Latrine or another	120	72	60.0	
Crowding at home				
No	41	26	63.4	0.05
Semi-crowded	55	27	49.1	
Overcrowded	29	22	75.9	
Education of the head of family				
7 years or more	17	10	58.8	0.52
4 - 6 years	53	29	54.7	
Up to 3 years	55	36	65.5	

significant.

Ethics statement

The Ethical Committee of the General Hospital of the Secretary of Health in Durango City approved this project. The purpose and procedures of this study were explained to all miners examined. A written informed consent was obtained from each participant.

Results

Anti-*T. gondii* IgG antibodies were detected in 75 (60.0%) of 125 miners and in 55 (22.0%) of 250 controls. Seroprevalence of anti-*T. gondii* IgG antibodies was significantly higher in miners than in controls (OR = 5.31; 95% CI: 3.33 - 8.47;

Table 2. Bivariate Analysis of Selected Behavioral Characteristics and Infection With *T. gondii* in Miners

Characteristics	No. of subjects tested	Prevalence of *T. gondii* infection		P value
		No.	%	
Cleaning cat excrement				
Yes	43	21	48.8	0.06
No	82	54	65.9	
Raising farm animals				
Yes	99	61	61.6	0.47
No	26	14	53.8	
National trips				
Yes	77	43	55.8	0.23
No	48	32	66.7	
Goat meat consumption				
Yes	101	56	55.4	0.03
No	24	19	79.2	
Sheep meat consumption				
Yes	59	30	50.8	0.04
No	66	45	68.2	
Boar meat consumption				
Yes	94	61	64.9	0.05
No	31	14	45.2	
Chicken meat consumption				
Yes	124	75	60.5	0.40
No	1	0	0.0	
Turkey meat consumption				
Yes	65	32	49.2	0.01
No	60	43	71.7	
Pigeon meat consumption				
Yes	72	48	66.7	0.07
No	53	27	50.9	
Quail meat consumption				
Yes	39	26	66.7	0.30
No	86	49	57.0	
Rabbit meat consumption				
Yes	51	27	52.9	0.18
No	74	48	64.9	
Armadillo meat consumption				
Yes	60	38	63.3	0.46
No	65	37	56.9	
Iguana meat consumption				
Yes	24	16	66.7	0.45
No	101	59	58.4	
Badger meat consumption				
Yes	24	17	70.8	0.22
No	101	58	57.4	

Table 2. Bivariate Analysis of Selected Behavioral Characteristics and Infection With *T. gondii* in Miners - *(continued)*

Characteristics	No. of subjects tested	Prevalence of *T. gondii* infection		P value
		No.	%	
Degree of meat cooking				
Undercooked	6	5	83.3	0.39
Well done	117	68	58.1	
Raw dried meat				
Yes	85	46	54.1	0.06
No	39	28	71.8	
Chorizo consumption				
Yes	124	75	60.5	0.40
No	1	0	0.0	
Brain of cow consumption				
Yes	48	24	50.0	0.07
No	77	51	66.2	
Unwashed raw vegetables				
Yes	85	54	63.5	0.24
No	40	21	52.5	
Untreated water				
Yes	107	66	61.7	0.34
No	18	9	50.0	
Frequency of eating out of home				
Never	1	1	100.0	0.19
1 - 10 times a year	84	46	54.8	
> 10 times a year	40	28	70.0	
Alcohol consumption				
Yes	67	45	67.2	0.07
No	58	30	51.7	
Tobacco smoking				
Yes	57	39	68.4	0.07
No	68	36	52.9	

P < 0.001). Of the 75 anti-*T. gondii* IgG positive miners, 30 (40.0%) had anti-*T. gondii* IgG antibody levels higher than 150 IU/mL, 10 (13.3%) between 100 and 150 IU/mL, and 35 (46.7%) between 8 and 99 IU/mL. All seropositive controls had ≥ 8 IU/mL of anti-*T. gondii* IgG antibodies as determined by the qualitative test. However, we were unable to quantitate further for the specific IgG level in all 55 seropositive controls. IgG levels could be determined in only 36 of 55 seropositive controls; of these 27 (75.0%) had anti-*T. gondii* IgG antibody levels higher than 150 IU/mL, two (5.6%) between 100 and 150 IU/mL, and seven (19.4%) between 8 and 99 IU/mL. The frequency of individuals with high IgG levels was significantly higher in the controls compared to the cases group (P = 0.003).

Of the 75 miners seropositive for anti-*T. gondii* IgG antibodies, 39 (52.0%) were also positive for anti-*T. gondii* IgM antibodies compared to only eight (14.5%) of the 55 controls seropositive to anti-*T. gondii* IgG antibodies (P < 0.001). DNA of *T. gondii* was detected in 13 miners, and eight (61.5%) of them were positive for anti-*T. gondii* IgM antibodies.

The frequency of IgG was similar (P = 0.47) in participants working less than 1 year as a miner (62.5%) and those with 1 - 5 years (68.8%) or > 5 years (56.5%); the frequency of IgM was comparable (P = 0.92) in participants with less than 1 year of working as a miner (25.0%), those with 1 - 5 years (31.3%) or those with > 5 years (31.8%).

Concerning socio-demographic and housing characteristics (Table 1), bivariate analysis showed three characteristics potentially (P values ≤ 0.10) associated with *T. gondii* infection: birth place (P = 0.02), socioeconomic status (P = 0.04), and crowing at home (P = 0.05). Other socio-demographic and housing characteristics of miners including age, educational level, flooring at home, availability of potable water, form of elimination of excretes, and years of education of the head of

Table 3. Multivariate Analysis of Selected Characteristics of Miners and Their Association With *T. gondii* Infection

Characteristics	Odds ratio	95% confidence interval	P value
Birth place (Durango State)	3.44	1.09 - 10.7	0.03
Socioeconomic level (low)	2.05	0.73 - 5.71	0.17
Cleaning cat excrement (yes)	0.33	0.11 - 0.90	0.03
Goat meat consumption (yes)	0.16	0.03 - 0.76	0.02
Sheep meat consumption (yes)	1.10	0.34 - 3.48	0.88
Boar meat consumption (yes)	5.53	1.49 - 20.3	0.01
Turkey meat consumption (yes)	0.35	0.12 - 1.00	0.05
Pigeon meat consumption (yes)	2.20	0.80 - 5.98	0.12
Raw dried meat (yes)	0.45	0.15 - 1.32	0.15
Brain of cow consumption (yes)	0.50	0.18 - 1.32	0.16
Alcohol consumption (yes)	0.83	0.27 - 2.51	0.75
Tobacco use (yes)	2.40	0.85 - 6.73	0.10
Crowding			
Semi-crowded	2.78	0.86 - 8.93	0.09
Overcrowded	5.83	1.49 - 22.8	0.01

the family had P values > 0.10.

With respect to the clinical characteristics, all seropositive miners referred themselves as healthy. The frequency of *T. gondii* seropositivity was higher in miners without memory impairment (56/85, 65.9%) than in miners with memory impairment (18/39, 46.2%) (P = 0.03). Other clinical characteristics including history of lymphadenopathy, surgery, blood transfusion, impairments in reflexes, hearing and vision, frequent abdominal pain or headache, and dizziness showed no association with seropositivity. None of the miners had received an organ transplant.

Of the behavioral characteristics of the miners examined (Table 2), 10 variables had P values ≤ 0.10 in the bivariate analysis: cleaning cat excrement (P = 0.06), consumption of meat from goat (P = 0.03), sheep (P = 0.04), boar (P = 0.05), turkey (P = 0.01), and pigeon (P = 0.07), consumption of raw dried meat (P = 0.06), cow's brains (P = 0.07), alcohol consumption (P = 0.07), and tobacco smoking (P = 0.07).

Multivariate analysis of socio-demographic, housing, and behavioral characteristics of miners with P values ≤ 0.10 in the bivariate analysis (Table 3) showed that *T. gondii* seropositivity was positively associated with being born in Durango State (OR = 3.44; 95% CI: 1.09 - 10.7; P = 0.03), consumption of boar meat (OR = 5.53; 95% CI: 1.49 - 20.3; P = 0.01), and overcrowded homes (OR = 5.83; 95% CI: 1.49 - 22.8; P = 0.01), and seropositivity was negatively associated with cleaning cat excrement (OR = 0.33; 95% CI: 0.11 - 0.90; P = 0.03), and consumption of goat meat (OR = 0.16; 95% CI: 0.03 - 0.76; P = 0.02).

Discussion

Very little is known about the epidemiology of *T. gondii* infection in miners. To the best of our knowledge, the correlation of *T. gondii* infection with the occupation of miner has not been assessed by an age- and gender-matched case-control study design. Therefore, we performed an age- and gender-matched case-control study to investigate the association of *T. gondii* infection with the occupation of miner in the northern Mexican State of Durango.

Remarkably, we found that the prevalence of *T. gondii* exposure was significantly higher in miners than in controls. The seroprevalence found in miners in Durango, Mexico is higher than those reported in miners in other countries. A 7.7% prevalence of infection was found in coal miners in China using the indirect hemagglutination test [12]. In Ukraine, 37.7% of miners were seropositive for *T. gondii* using complement-fixation, passive hemagglutination, and intradermal toxoplasmin tests [13]. Furthermore, the high seroprevalence of *T. gondii* exposure found in miners (60.0%) is the highest seroprevalence reported in population groups in Durango State so far. Thus, seroprevalence found in miners is higher than the seroprevalences of *T. gondii* infection reported in adults in rural communities in Durango State (23.8%) [10], in schizophrenic patients (20%) [14], in waste pickers (21.1%) [15], inmates (21.1%) [16], and ethnic groups living in rural communities including Tepehuanos (22.4%) [17] and Huicholes (33.2%) [18]. In addition, the seroprevalence found in miners is higher than the weighted mean (19.27%) national seroprevalence of *T. gondii* infection found in Mexico [19]. It is not clear why miners had a higher seroprevalence of *T. gondii* exposure than age- and gender-matched controls which were also obtained from rural settings. Seroprevalence of infection with *T. gondii* increases with age as reported in general populations in rural [10] and urban [20] Durango. However, in the present study, no such increase in *T. gondii* exposure with age was observed, and a surprisingly high (61.5%) seroprevalence of *T. gondii* infec-

tion was already observed in the youngest miners aged 18 - 30 years old.

We searched for socio-demographic, work, housing and behavioral characteristics to investigate the high seroprevalence of *T. gondii* in miners. Duration in the activity did not correlate with *T. gondii* exposure. Even miners with less than 1 year as a miners had a high seroprevalence of infection with *T. gondii*. Multivariate analysis showed that *T. gondii* exposure was positively associated with being born in Durango State. This result was unexpected since *T. gondii* exposure has been repeatedly associated with the characteristic of being born out of Durango State in diverse cohorts including the general population in Durango City [20], inmates [16], patients with vision and hearing impairments, cancer, HIV, or undergoing hemodialysis [21], female sex workers [22], elderly people [23], patients with heart diseases [24], and people applying for medical certificates [25]. The association of *T. gondii* infection with being born in Durango State found in this study likely indicates that infection was acquired in Durango State. In fact, traveling did not increase the prevalence of infection with *T. gondii* in miners. Furthermore, multivariate analysis showed that infection with *T. gondii* was associated with consumption of boar meat, and living in an overcrowded home. These characteristics may have contributed to the high seroprevalence of *T. gondii* infection in miners. Consumption of boar meat was associated with *T. gondii* seropositivity in several populations in the region with lower seroprevalence than miners including patients with work accidents [26], elderly people [23], and the general population in Durango City [20]. Concerning the association of infection with *T. gondii* and living in an overcrowded home, this is the first time we found such association in our studies of infection with *T. gondii* in the region. Living in an overcrowded area has been considered as a contributing factor for infection with *T. gondii* in pregnant women in Nigeria [27]. It is not clear why overcrowding influenced the seroprevalence of *T. gondii* infection in that study, but other factors for infection including poor sanitation and contamination of environment with cat excrement were also present [27]. In addition, in the Third National Health and Nutrition Examination Survey in the USA (1988 - 1994), researchers found that risk for *T. gondii* infection increased in those who lived in crowded conditions [28]. On the other hand, we found that infection with *T. gondii* was negatively associated with cleaning cat excrement, and consumption of goat meat. These factors have been suggested as risks for infection with *T. gondii* exposure by others [29, 30].

The present study has some limitations. The sample size is small, and we studied miners working in only one mine. Further studies should include a larger sample size and sample miners of more than one mine. A high frequency (61.5%) of positive *T. gondii* PCR assays among miners seropositive for anti-*T. gondii* IgM antibodies was found. Therefore, further research on the epidemiology of acute cases of *T. gondii* infection in miners should be conducted.

We conclude that miners represent a risk group for infection with *T. gondii*. This is the first age- and gender-matched study on the association of *T. gondii* infection and the occupation of miner. Further studies to confirm our results are needed.

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INFLUENCE OF *TOXOPLASMA GONDII* INFECTION ON SYMPTOMS AND SIGNS OF PREMENSTRUAL SYNDROME: A CROSS-SECTIONAL STUDY

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Infection with *Toxoplasma gondii* in brain may cause some symptoms that resemble those in women with premenstrual syndrome. To determine the association of *T. gondii* infection with symptoms and signs of premenstrual syndrome, we examined 489 women aged 30–40 years old. Sera of participants were analyzed for the presence of anti-*Toxoplasma* IgG and IgM antibodies using enzyme-linked immunoassays (EIA) and *T. gondii* DNA by polymerase chain reaction (PCR).

Anti-*T. gondii* IgG antibodies were found in 38 (7.8%) of the women studied. Anti-*T. gondii* IgM antibodies were found in 13 (34.2%) of the 38 IgG seropositive women. Logistic regression showed two variables associated with seropositivity to *T. gondii:* presence of diarrhea (odds ratio [OR] = 6.10; 95% confidence interval [CI]: 1.37–27.85; $P = 0.01$) and weight gain (OR = 2.89; 95% CI: 1.37–6.07; $P = 0.005$), and two variables associated with high (>150 IU/ml) levels of IgG against *T. gondii:* presence of diarrhea (OR = 7.40; 95% CI: 1.79–30.46; $P = 0.006$) and abdominal inflammation (OR = 3.38; 95% CI: 1.13–10.10; $P = 0.02$). Positivity to EIA IgG and PCR was positively associated with obesity and negatively associated with joint pain by bivariate analysis.

Our study for the first time reveals a potential association of *T. gondii* infection with clinical manifestations of premenstrual syndrome.

Keywords: *Toxoplasma gondii,* seroprevalence, premenstrual syndrome, cross-sectional study

Introduction

It is estimated that about one third of humanity is infected with the protozoan parasite *Toxoplasma gondii* [1, 2]. Infection with *T. gondii* is zoonotic, and it is most frequently acquired by the ingestion of raw or undercooked meat of *T. gondii*-infected animals containing tissue cysts, or ingestion of food or water contaminated with *T. gondii* oocysts shed by cats [3, 4]. Other routes of *T. gondii* infection are vertical [5], organ transplantation [6], and blood transfusion [7]. Most infections with *T. gondii* are asymptomatic [3]. However, some infected individuals develop clinical manifestations of the disease called toxoplasmosis [5]. Individuals suffering from toxoplasmosis may have involvement of lymph nodes, eyes, or central nervous system [3, 8]. A life-threatening toxoplasmosis

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may occur in immunocompromised patients [9]. Infection with *T. gondii* has been linked to psychiatric illnesses, i.e., schizophrenia [10, 11], obsessive–compulsive disorder [10], intermittent explosive disorder [12], depression [13], and generalized anxiety disorder [14]. A number of general symptoms of toxoplasmosis have been described including headache [15, 16], pain and weakness of muscles [16–18], fatigue [16, 19, 20], difficulty concentrating [19], and confusion [13].

Premenstrual syndrome is characterized by recurrent affective, physical, and behavioral symptoms that develop during the luteal menstrual cycle and disappear within a few days of menstruation [21, 22]. A severe form of this syndrome is called premenstrual dysphoric disorder [22, 23]. Clinical manifestations of premenstrual syndrome include fatigue, impaired concentration [24, 25], confusion [24], headache [26], and depression [27]. These clinical features are also observed in toxoplasmosis. It is possible that infection with *T. gondii* in brain might cause or influence some symptoms in women during the premenstrual period. In a recent study, infection with *T. gondii* was associated with out of control feeling or overwhelmed in women suffering from premenstrual dysphoric disorder [28]. However, it is unknown whether *T. gondii* infection might influence symptoms in premenstrual syndrome. Symptoms of premenstrual syndrome might be not only hormonal but also nervous in nature. Infection with *T. gondii* in brain might be linked to clinical manifestations in premenstrual syndrome as occurred in menopause [29]. Since the link of *T. gondii* infection and clinical manifestations of premenstrual syndrome has not been investigated in the past, we investigated the association of *T. gondii* infection with clinical characteristics of premenstrual syndrome in women in the northern Mexican city of Durango.

Materials and methods

Study design and study population

We performed a cross-sectional study of 489 women who attended general consultations in two public primary healthcare centers: Centro de Salud #2 of the Secretary of Health (n = 327) and Clinic of Family Medicine of the Institute of Security and Social Services of State Workers (n = 162) in Durango City, Mexico. All women were examined from February to April 2016. Inclusion criteria for enrollment were women aged 30–40 years old who accepted to participate in the study. Socioeconomic status and occupation of the women were not restrictive criteria for enrollment. Pregnancy was an exclusion criterion. Mean age in women studied was 35.27 ± 3.47.

Clinical characteristics of women

We used a face-to-face questionnaire to record the symptoms and signs of premenstrual syndrome in the women studied. Clinical data studied were presence of irregular periods, severity of menstruation, suffering from mental illness, vaginal infections, thyroid disease, obesity, arterial hypertension, sleep problems, fatigue, memory lapses, difficulty concentrating, confusion, judgment problems, mood changes, low self-esteem, depression, guilty feeling, increase of fears, panic attacks, anxiety, tension, nervousness, irritability, aggressiveness, lack of interest in daily activities, lack of interest in social relations, out of control feeling or overwhelmed, reduced tolerance to noises and lights, dizziness, headache, migraine, allergy, breast pain, bouts of rapid heartbeat, decrease in muscle power, joint pain, low back pain, muscle tension, clumsiness, tingling extremities, electric shock sensation, bruises, painful periods, edema in ankles, hands or feet, decreased libido, increased libido, dyspareunia, abdominal bloating, gas, abdominal pain, constipation, diarrhea, nauseas, abdominal inflammation, appetite disturbance, desire to eat certain food or eat a lot, weight gain, presence of acne, presence of herpes labialis, and respiratory problems.

*Detection of anti-*T. gondii *antibodies*

We obtained a serum sample from each woman. Sera were frozen at −20 °C until analyzed. Anti-*T. gondii* IgG antibodies were detected in sera with the commercially available enzyme immunoassay (EIA) kit "*Toxoplasma* IgG" (International Immuno-Diagnostics, Foster City, CA, USA). Anti-*T. gondii* IgG antibody levels were expressed as International Units (IU)/ml, and a cut off of ≥ 8 IU/ml was used for seropositivity. Sera with anti-*T. gondii* IgG antibodies were further analyzed for anti-*T. gondii* IgM antibodies by the commercially available EIA "*Toxoplasma* IgM" kit (Diagnostic Automation Inc., Calabasas, CA, USA). All assays were performed according to the manufacturer's instructions.

DNA extraction and T. gondii *polymerase chain reaction*

Women with positive EIA for *T. gondii* IgG antibodies were further examined to detect *T. gondii* DNA by nested polymerase chain reaction (PCR). Extraction of DNA was performed from whole blood according to a protocol described by Iranpour and Esmailizadeh (http://www.protocol-online.org/prot/Protocols/Rapid-Extraction-of-High-Quality-DNA-from-Whole-Blood-Stored-at-4-C-for-Long-Period-4175.html). We used a PCR protocol and two pairs of primer directed against the B1 gene of *T. gondii* as described elsewhere [30]. This protocol was previously tested and showed high specificity and sensitivity: 0.01 to 0.02 fg of the target DNA in the presence of 1 µg of contaminating negative human DNA was detected by PCR [31]. PCR products were electrophoresed with agarose gels, stained with ethidium bromide, and visualized by ultraviolet transillumination.

Statistical analysis

Analysis of results was performed by using the following software: SPSS 15.0 (SPSS Inc. Chicago, Illinois), Microsoft Excel, and Epi Info 7. For calculation of the sample size, we used: a) a reference seroprevalence of 6.1% [32] as the expected frequency for the factor under study, b) 200,000 as the population size from which the sample was selected, c) a 2.2% of confidence limits, and d) a 95% confidence level. The result of the sample size calculation was 454 subjects. We assessed the association of *T. gondii* seropositivity and the clinical characteristics of women with the Pearson's chi-squared test or the two-tailed Fisher's exact test (when values were small). We included in the multivariate analysis only variables with a *P* value ≤0.10 obtained in the bivariate analysis. Odds ratio (OR) and 95% confidence interval (CI) were calculated by multivariate analysis using logistic regression with the Enter method. Statistical significance was set at a *P* value <0.05.

Ethical aspects

This study was approved by the Ethics Committee of the General Hospital of the Secretary of Health in Durango City, Mexico. Participation was voluntary, and the purpose and procedures of this study were explained to all participants. Furthermore, a written informed consent was obtained from each participant.

Results

Anti-*T. gondii* IgG antibodies were found in 38 (7.8%) of the 489 women studied. Of the 38 anti-*T. gondii* IgG positive women, 22 (57.9%) had IgG levels >150 IU/ml, 1 (2.6%) between 100 and 150 IU/ml, and 15 (39.5%) between 8 and 99 IU/ml. Anti-*T. gondii* IgM antibodies were found in 13 (34.2%) of the 38 IgG seropositive women. DNA of *T. gondii* was detected in six (15.8%) of the 38 women with IgG antibodies against *T. gondii*.

Seropositive women showed from four to 41 (mean: 15.1 ± 8.5) signs or symptoms of premenstrual syndrome. Seronegative women (n = 451) had from one to 45 (mean: 16.7 ± 9.1) signs or symptoms of premenstrual syndrome. Mean number of signs or symptoms in seropositive women was similar to that found in seronegative women (P = 0.28).

Bivariate analysis of clinical characteristics of premenstrual syndrome and IgG seropositivity to *T. gondii* showed ten variables with a *P* value less than 0.10: confusion, allergy, low back pain, tingling extremities, electric shock sensation, increased libido, abdominal bloating, gas, diarrhea, and weight gain. Other clinical characteristics of premenstrual syndrome showed *P* values equal to or higher than 0.10 by bivariate analysis. *Table 1* shows results of bivariate analysis of a selection of clinical data of premenstrual syndrome and IgG seropositivity to *T. gondii*. Further analysis by logistic regression of variables with *P* value less than 0.10 obtained by bivariate analysis

Table 1. Results of bivariate analysis of a selection of premenstrual clinical characteristics of women and IgG seropositivity to *T. gondii*

	Women tested	Prevalence of *T. gondii* infection		*P* value
Characteristic	No.	No.	%	
Obesity				
Yes	192	18	9.4	0.28
No	297	20	6.7	
Arterial hypertension				
Yes	46	6	13.0	0.14
No	441	31	7.0	
Confusion				
Yes	99	3	3	0.04
No	389	35	9	
Irritability				
Yes	240	15	6.3	0.21
No	249	23	9.2	
Reduced tolerance to noises and lights				
Yes	131	6	4.6	0.11
No	358	32	8.9	
Dizziness				
Yes	172	9	5.2	0.12
No	317	29	9.1	

Table 1. *(cont'd)*

	Women tested	Prevalence of *T. gondii* infection		*P* value
Characteristic	No.	No.	%	
Headache				
Yes	278	23	8.3	0.63
No	211	15	7.1	
Migraine				
Yes	124	7	5.6	0.30
No	365	31	8.5	
Allergy				
Yes	118	5	4.2	0.10
No	371	33	8.9	
Breast pain				
Yes	234	15	6.4	0.28
No	255	23	9	
Bouts of rapid heart beat				
Yes	115	5	4.3	0.11
No	373	33	8.8	
Decrease in muscle power				
Yes	176	10	5.7	0.19
No	313	28	8.9	
Joint pain				
Yes	217	13	6	0.23
No	270	24	8.9	
Low back pain				
Yes	308	18	5.8	0.03
No	181	20	11	
Tingling extremities				
Yes	200	10	5	0.05
No	289	28	9.7	
Electric shock sensation				
Yes	131	5	3.8	0.04
No	358	33	9.2	
Edema in ankles, hands, or feet				
Yes	111	7	6.3	0.49
No	375	31	8.3	
Decreased libido				
Yes	151	9	6	0.30
No	334	29	8.7	
Increased libido				
Yes	34	0	0	0.09
No	449	38	8.5	
Abdominal bloating				
Yes	108	6	5.6	0.00
No	381	32	8.4	
Gas				
Yes	30	5	16.7	0.07
No	459	33	7.2	

Table 1. *(cont'd)*

	Women tested	Prevalence of *T. gondii* infection		*P* value
Characteristic	No.	No.	%	
Constipation				
Yes	123	6	4.9	0.16
No	366	32	8.7	
Diarrhea				
Yes	14	3	21.4	0.08
No	475	35	7.4	
Abdominal inflammation				
Yes	48	6	12.5	0.24
No	441	32	7.3	
Weight gain				
Yes	219	23	10.5	0.03
No	267	14	5.2	

showed that only two variables were associated with seropositivity to *T. gondii*: presence of diarrhea (OR = 6.10; 95% CI: 1.37–27.85; $P = 0.01$) and weight gain (OR = 2.89; 95% CI: 1.37–6.07; $P = 0.005$) *(Table 2)*.

Bivariate analysis of clinical characteristics of premenstrual syndrome and high (>150 IU/ml) IgG levels to *T. gondii* showed only six variables with a *P* value less than 0.10: low self-esteem, irritability, low back pain, tingling extremities, diarrhea, and abdominal inflammation. Further analysis by logistic regression of these variables with *P* values less than 0.10 obtained by bivariate analysis showed that two variables were associated with high levels of IgG against *T. gondii*: presence of diarrhea (OR = 7.40; 95% CI: 1.79–30.46; $P = 0.006$) and abdominal inflammation (OR = 3.38; 95% CI: 1.13–10.10; $P = 0.02$) *(Table 3)*.

With respect to the association of premenstrual clinical manifestations and seropositivity of both IgG and IgM anti-*T. gondii,* bivariate analysis showed no significant associations, and only the variables tingling extremities and diarrhea showed borderline ($P = 0.05$) associations.

Table 2. Multivariate analysis of selected premenstrual clinical characteristics of women and their association with *T. gondii* infection

Characteristic	Odds ratio	95% confidence interval	*P* value
Confusion	0.38	0.11–1.34	0.13
Allergy	0.50	0.18–1.38	0.18
Low back pain	0.48	0.23–1.02	0.05
Tingling extremities	0.78	0.33–1.86	0.58
Electric shock sensation	0.52	0.17–1.58	0.25
Abdominal bloating	0.89	0.33–2.34	0.81
Gas	2.60	0.79–8.57	0.11
Diarrhea	6.10	1.37–27.85	0.01
Weight gain	2.89	1.37–6.07	0.005

Table 3. Multivariate analysis of selected premenstrual characteristics and their association with high (>150 IU/ml) levels of IgG to *T. gondii*

Characteristic	Odds ratio	95% confidence interval	*P* value
Low self-esteem	0.58	0.19–1.74	0.33
Irritability	0.67	0.24–1.86	0.44
Low back pain	0.53	0.20–1.36	0.18
Tingling extremities	0.62	0.21–1.81	0.38
Diarrhea	7.40	1.79–30.46	0.006
Abdominal inflammation	3.38	1.13–10.10	0.02

To avoid bias and due to a small number of cases with IgM seropositivity, no further regression analysis with these variables was performed.

Concerning the results of the positivity to both IgG antibodies against *T. gondii* and DNA of *T. gondii* by PCR, women with obesity showed a significantly ($P = 0.03$) higher prevalence of *T. gondii* (5/192: 2.6%) than women without obesity (1/297: 0.3%) whereas women with joint pain showed a significantly ($P = 0.03$) lower prevalence of *T. gondii* (0/217) than women without joint pain (6/270: 2.2%).

Discussion

Premenstrual syndrome has a number of signs and symptoms also observed in toxoplasmosis. Therefore, we hypothesized that *T. gondii* infection may have an influence on clinical manifestations of premenstrual syndrome. As far as we know, the association between *T. gondii* infection and signs and symptoms of premenstrual syndrome has not been assessed yet. Therefore, this study aimed to determine whether infection with *T. gondii* was associated with clinical characteristics of premenstrual syndrome in women in Durango City, Mexico. We found that women seropositive for *T. gondii* had a similar mean number of signs or symptoms of premenstrual syndrome than seronegative women. Results suggest that infection with *T. gondii* does not influence on the number of clinical manifestations of premenstrual syndrome in general. However, logistic regression showed in particular that infection with *T. gondii* is associated with specific clinical characteristics of premenstrual syndrome. Thus, results suggest that *T. gondii* infection may influence qualitatively on clinical manifestations of premenstrual syndrome. Remarkably, both IgG seropositivity to *T. gondii* and high levels of IgG against *T. gondii* were associated with the presence of diarrhea. It is not clear why infection with *T. gondii* was associated with diarrhea during the premenstrual period. Diarrhea is a well-known clinical sign included within the physical features of premenstrual syndrome [33]. In a Chinese study about prevalence of premenstrual syndrome in women at reproductive age, researchers found diarrhea as the fourth most frequent clinical characteristic in premenstrual syndrome just after irritation, depression, and anxiety [33]. In addition, in a prevalence study about the menstrual cycle and its effect on inflammatory bowel disease and irritable bowel syndrome, Kane et al. found that women with Crohn's disease were more likely to report increased gastrointestinal symptoms during menstruation, being diarrhea the clinical feature reported most often [34]. In a recent study, Zhang et al. reported that Chinese women suffering from both diarrhea-predominant irritable bowel syndrome and premenstrual syndrome had more severe bowel symptoms [35]. On the other hand, infection with *T. gondii* may lead to diarrhea in humans and animals [36]. Presence of diarrhea in *T. gondii* infected individuals has been unusually reported. However, the link between infection with *T. gondii* and diarrhea in humans has been scantly studied. A case of gastric toxoplasmosis with diarrhea in a man with acquired immunodeficiency syndrome was reported [37]. Similarly, a case of toxoplasmic colitis with diarrhea where microorganisms were identified in the colonic mucosa and confirmed by immunohistochemistry was reported [38]. In animals, severe or fatal toxoplasmosis cases with diarrhea have been reported in cats [39, 40] and a valley quail [41]. It is unclear how frequent diarrhea occurs in immunocompromised and immunocompetent subjects. We may hypothesize that *T. gondii* may affect intestines of women during the premenstrual period perhaps under a hormonal influence leading to diarrhea. It is also possible that *T. gondii* causes diarrhea by affecting enteric neurons. Experiments in rats have shown that infection with *T. gondii* causes changes in myenteric neurons of the jejunum, i.e., atrophy of myenteric neurons along with increased weight gain in rats at 30 days of infection, or hypertrophy of myenteric neurons along with normal weight gain in rats at 90 days after infection [36]. Interestingly, IgG seropositivity to *T. gondii* was associated with weight gain. It is not clear why women who have gained weight had a higher seroprevalence of *T. gondii* infection that those without weight gain. Experimental infections of *T. gondii* have showed weight gain in rats after 30 days of infection [36]. In humans, *T. gondii* infection has been associated with weight gain in pregnant women [42]. In addition, both *T. gondii* seroprevalence and high IgG anti-*T. gondii* antibody levels have been associated with obesity [43]. In fact, results of the positivity to both IgG antibodies against *T. gondii* and DNA of *T. gondii* in this study showed that women with obesity had a significantly higher prevalence of *T. gondii* than women without obesity. On the other hand, women with joint pain showed a significantly lower prevalence of *T. gondii* than women without joint pain. This finding suggests that *T. gondii* was not an important factor for joint pain in the women studied.

Logistic regression analysis also showed that high levels of IgG against *T. gondii* were associated with abdominal inflammation. *T. gondii* may cause inflammation in organs and tissues in the abdomen, as observed in experimental infections in mice [44]. Therefore, the presence of this clinical feature may suggest an active immune reaction against *T. gondii* in abdomen.

Limitations of our study included small sample size of the women studied and a low prevalence of IgG, IgM, and PCR positivity. However, strengths of our study include that women were studied from two health centers of Durango City and that we used detection of DNA of *T. gondii* to increase the evidence of *T. gondii* exposure.

Conclusions

The present study for the first time points towards an association of *T. gondii* infection with clinical manifestations of premenstrual syndrome, i.e., physical symptoms. Re-

sults warrant further research of the role of *T. gondii* on clinical manifestations of premenstrual syndrome.

Competing interests

The authors declare that no competing interests exist.

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Toxoplasma gondii Infection and Premenstrual Dysphoric Disorder: A Cross-Sectional Study

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Abstract

Background: Premenstrual dysphoric disorder is a severe form of premenstrual syndrome. The influence of *Toxoplasma gondii (T. gondii)* infection on clinical features in women with this disorder has not been studied. Therefore, we determined the association of *T. gondii* infection with symptoms and signs in women suffering from premenstrual dysphoric disorder.

Methods: We performed a cross-sectional study of 151 women suffering from premenstrual dysphoric disorder. Anti-*Toxoplasma* IgG and IgM antibodies were detected in sera of the participants using enzyme-linked immunoassays (EIAs). In addition, *T. gondii* DNA was detected in whole blood of IgG seropositive participants using polymerase chain reaction. We obtained the clinical data of women with the aid of a questionnaire. The association of *T. gondii* infection with clinical characteristics of women was assessed by bivariate and multivariate analyses.

Results: Anti-*T. gondii* IgG antibodies were found in 10 (6.6%) of the 151 women studied. Of the 10 IgG seropositive women, four (40.0%) were positive for anti-*T. gondii* IgM antibodies, and one (10.0%) for *T. gondii* DNA. Mean number (25.8 ± 7.58) of premenstrual clinical characteristics in seropositive women was similar to that (29.22 ± 9.13) found in seronegative women (P = 0.25). Logistic regression showed that seropositivity to *T. gondii* was negatively associated with difficulty concentrating (OR: 0.18; 95% CI: 0.03 - 0.91; P = 0.03), and positively associated with out of control feeling or overwhelmed (OR: 9.00; 95% CI: 1.32 - 62.00; P = 0.02).

Conclusions: Results of this first study on the association of *T. gondii* infection and clinical characteristics of premenstrual dysphoric disorder suggest that this infection might be linked to some symptoms of this disorder. We report for the first time the association of *T. gondii* infection and out of control feeling or overwhelmed. Results warrant for further research on the role of *T. gondii* in premenstrual dysphoric disorder.

Keywords: *Toxoplasma gondii*; Seroprevalence; Premenstrual dysphoric disorder; Cross-sectional study

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Introduction

The coccidian parasite *Toxoplasma gondii (T. gondii)* causes infections in humans worldwide [1]. Cats are the definitive host of *T. gondii*, whereas humans and other warm-blooded animals are intermediate hosts [2]. Most infections with *T. gondii* in humans are acquired by ingestion of food or water contaminated with *T. gondii* oocysts shed by cats, or by the ingestion of raw or undercooked meat containing tissue cysts [3]. Less frequently, infection with *T. gondii* may occur by organ transplantation [4], and blood transfusion [5]. In addition, primary infection with *T. gondii* during pregnancy may lead to vertical transmission and congenital disease [3, 6]. Infections with *T. gondii* are usually asymptomatic [3]. Subjects with clinical manifestations of infection (toxoplasmosis) may present with disease in eyes, lymph nodes and central nervous system [3, 7, 8]. Toxoplasmosis is particularly severe in immunocompromised individuals [9]. Common symptoms of toxoplasmosis include fatigue, headache, muscle aches, and difficulty concentrating [10]. Furthermore, infection with *T. gondii* has been associated with a number of psychiatric disorders including depression [11], schizophrenia [11, 12], im-

pulsive aggression [13], generalized anxiety disorder [14], and suicide attempts [15].

Premenstrual dysphoric disorder is a severe form of premenstrual syndrome with serious psychological symptoms [16]. This disorder is characterized by cognitive-affective symptoms that appear in a cyclic manner during the premenstrual period [17]. This illness has been recently designated as a disorder in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (http://www.dsm5.org/Pages/Default.aspx). Prevalence of premenstrual dysphoric disorder varies from 1.3% to 8% [18, 19]. Symptoms of this disorder significantly impair daily functioning [20, 21], and its etiology is unclear [16, 18, 20]. It raises the question whether *T. gondii* infection plays a role in this disorder as it does in other psychiatric disorders. To our knowledge, the link of *T. gondii* infection and premenstrual dysphoric disorder has not been studied. Therefore, we sought to determine the association of *T. gondii* infection with clinical characteristics of premenstrual dysphoric disorder in a sample of women in Durango City, Mexico.

Materials and Methods

Study design and population studied

Through a cross-sectional study, we examined 151 women with premenstrual dysphoric disorder. Women studied were enrolled in two public primary healthcare centers: Centro de Salud #2 of the Secretary of Health (n = 78), and Clinic of Family Medicine of the Institute of Security and Social Services of State Workers (n = 73) in the northern Mexican city of Durango. Participants were examined from February to April 2016. Inclusion criteria for enrollment were women suffering from premenstrual dysphoric disorder, aged 30 - 40 years old, and who accepted to participate in the study. Diagnosis of premenstrual dysphoric disorder was made according to the DSM-5 criteria [22]. Occupation, civil status, and socioeconomic level of women were not restrictive criteria for enrollment. Pregnant women were not included in the study. Mean age in women examined was 35.52 ± 3.59.

Clinical data of the women studied

Signs and symptoms of women were obtained with the aid of a questionnaire. In total, 59 clinical characteristics of women were recorded including presence of irregular periods, severity of menstruation, vaginal infections, painful periods, fatigue, memory lapses, difficulty concentrating, confusion, judgment problems, suffering from mental illness, mood changes, low self-esteem, depression, guilty feeling, increase of fears, panic attacks, anxiety, tension, nervousness, irritability, aggressiveness, lack of interest in daily activities, lack of interest in social relations, out of control feeling or overwhelmed, reduced tolerance to noises and lights, dizziness, headache, migraine, allergy, breast pain, arterial hypertension, bouts of rapid heartbeat, decrease in muscle power, joint pain, low back pain, muscle tension, clumsiness, tingling extremities, electric shock sensation, bruises, edema in ankles, hands or feet, decreased libido, increased libido, dyspareunia, abdominal bloating, gas, abdominal pain, constipation, diarrhea, nauseas, abdominal inflammation, appetite disturbance, desire to eat certain food or eat a lot, weight gain, respiratory problems, presence of acne, presence of herpes labialis, sleep problems, thyroid disease, and obesity.

Detection of anti-*T. gondii* IgG and IgM antibodies

A serum sample from each woman was obtained. Serum samples were stored at -20 °C until analyzed. Anti-*T. gondii* IgG antibodies were detected in serum samples with the commercially available enzyme immunoassay (EIA) kit "*Toxoplasma* IgG" (Diagnostic Automation/Cortez Diagnostics Inc., Woodland Hills, CA, USA). This kit was also used to quantitate the IgG levels. Anti-*T. gondii* IgG antibody levels were expressed as International Units (IU)/mL. A cut off of ≥ 8 IU/mL of IgG was used for seropositivity. Sera reactive for anti-*T. gondii* IgG antibodies were further analyzed for anti-*T. gondii* IgM antibodies by the commercially available EIA "*Toxoplasma* IgM" kit (Diagnostic Automation/Cortez Diagnostics Inc.). All EIAs were performed following the instructions of the manufacturer.

Detection of T. gondii DNA

Whole blood of women with anti-*T. gondii* IgG antibodies was analyzed to detect *T. gondii* DNA by nested-polymerase chain reaction (PCR). DNA of whole blood was extracted following a protocol describe elsewhere (http://www.protocol-online.org/prot/Protocols/Rapid-Extraction-of-High-Quality-DNA-from-Whole-Blood-Stored-at-4-C-for-Long-Period-4175.html). Primers directed against the B1 gene of *T. gondii* and a PCR protocol previously described [23] were used. Sensitivity and specificity of this test were previously examined [24]. Amplification products were analyzed by electrophoresis using 2% agarose gels, stained with ethidium bromide, and visualized using ultraviolet illumination.

Statistical analysis

Results were analyzed with the aid of the software SPSS 15.0 (SPSS Inc. Chicago, IL), Microsoft Excel, and Epi Info 7. For calculation of the sample size, we used: 1) a reference seroprevalence of 6.1% [25] as the expected frequency for the factor under study, 2) 100,000 as the population size from which the sample was selected, 3) a 4.0% of confidence limits, and 4) a 95% confidence level. The result of the sample size calculation was 137 subjects. The association of *T. gondii* infection and the clinical characteristics of women was assessed with the Pearson's Chi-squared test or the two-tailed Fisher's exact test (when values were five or less). In the multivariate analysis, we included only variables with a P value ≤ 0.20 obtained

Table 1. Results of Bivariate Analysis of a Selection of Clinical Data and Seropositivity to *T. gondii*

Characteristics	No. of women tested	Prevalence of *T. gondii* infection		P value
		No.	%	
Irregular periods				
Yes	100	9	9	0.16
No	50	1	2	
Sleep problems				
Yes	85	4	4.7	0.33
No	66	6	9.1	
Difficulty concentrating				
Yes	109	4	3.7	0.02
No	41	6	14.6	
Judgment problems				
Yes	59	2	3.4	0.31
No	91	8	8.8	
Mood changes				
Yes	145	9	6.2	0.29
No	5	1	20	
Low self-esteem				
Yes	114	5	4.4	0.06
No	37	5	13.5	
Guilty feeling				
Yes	80	3	3.8	0.19
No	71	7	9.9	
Increase of fears				
Yes	83	3	3.6	0.11
No	68	7	10.3	
Anxiety				
Yes	103	5	4.9	0.29
No	48	5	10.4	
Tension				
Yes	111	6	5.4	0.45
No	40	4	10	
Nervousness				
Yes	119	7	5.9	0.44
No	32	3	9.4	
Irritability				
Yes	124	7	5.6	0.38
No	27	3	11.1	
Lack of interest in social relations				
Yes	92	8	8.7	0.31
No	59	2	3.4	
Out of control feeling or overwhelmed				
Yes	86	8	9.3	0.18
No	65	2	3.1	

Table 1. Results of Bivariate Analysis of a Selection of Clinical Data and Seropositivity to *T. gondii* - *(continued)*

Characteristics	No. of women tested	Prevalence of *T. gondii* infection		P value
		No.	%	
Reduced tolerance to noises and lights				
Yes	89	4	4.5	0.31
No	62	6	9.7	
Joint pain				
Yes	96	8	8.3	0.32
No	55	2	3.6	
Low back pain				
Yes	117	9	7.7	0.45
No	34	1	2.9	
Tingling extremities				
Yes	96	4	4.2	0.17
No	55	6	10.9	
Decreased libido				
Yes	81	7	8.6	0.34
No	68	3	4.4	
Dyspareunia				
Yes	42	1	2.4	0.28
No	108	9	8.3	
Presence of acne				
Yes	85	4	4.7	0.33
No	65	6	9.2	

*Sum may not add up to 151 because of missing values.

in the bivariate analysis. We calculated odds ratio (OR) and 95% confidence interval (CI) using logistic regression with the Enter method. A P value less than 0.05 was considered statistically significant.

Ethical aspects

This study was approved by the Ethics Committees of the General Hospital of the Secretary of Health, and the Institute of Security and Social Services of State Workers in Durango City, Mexico. Participation of women was voluntary. The purpose and procedures of this study were explained to all participants, and a written informed consent was obtained from all of them.

Results

Anti-*T. gondii* IgG antibodies were found in 10 (6.6%) of the 151 women with premenstrual dysphoric disorder studied. Of the 10 anti-*T. gondii* IgG positive women, five (50.0%) had IgG levels > 150 IU/mL, two (20.0%) between 100 and 150 IU/mL, and three (30.0%) between 8 and 99 IU/mL. Anti-*T. gondii* IgM antibodies were found in four (40.0%) of the 10 IgG seropositive women. DNA of *T. gondii* was detected in one (10.0%) of the 10 women with IgG antibodies against *T. gondii*. IgG levels in this women were 146 IU/mL.

Women seropositive to anti-*T. gondii* IgG antibodies showed from 13 to 40 (mean: 25.8 ± 7.58) signs or symptoms, whereas seronegative women (n = 141) had from 7 to 48 (mean: 29.22 ± 9.13) signs or symptoms. Mean number of clinical characteristics in seropositive women was similar to that found in seronegative women (P = 0.25).

Concerning clinical characteristics, bivariate analysis showed seven variables potentially ($P \leq 0.20$) associated with IgG seropositivity to *T. gondii*: irregular periods, difficulty concentrating, low self-esteem, guilty feeling, increase of fears, out of control feeling or overwhelmed, and tingling extremities. Other clinical characteristics studied showed P values higher than 0.20 by bivariate analysis. Results of bivariate analysis of a selection of clinical characteristics of women and IgG seropositivity to *T. gondii* are shown in Table 1. Further analysis by logistic regression of variables with $P \leq 0.20$ obtained by bivariate analysis showed that seropositivity to *T. gondii* was negatively associated with difficulty concentrating (OR: 0.18; 95% CI: 0.03 - 0.91; P = 0.03), and positively associated with out of control feeling or overwhelmed (OR: 9.00; 95% CI: 1.32 - 62.00; P = 0.02) (Table 2).

Bivariate analysis showed that the prevalence of high (> 150 IU/mL) IgG levels to *T. gondii* was significantly (P =

Table 2. Results of the Multivariate Analysis of Clinical Characteristics and IgG Seropositivity to *T. gondii*

Characteristics	Odds ratio	95% confidence interval	P value
Irregular periods	7.91	0.75 - 82.5	0.08
Difficulty concentrating	0.18	0.03 - 0.91	0.03
Low self-esteem	0.48	0.08 - 2.57	0.39
Guilty feeling	0.45	0.08 - 2.46	0.36
Increase of fears	0.58	0.11 - 3.10	0.53
Out of control feeling or overwhelmed	9.00	1.32 - 62.0	0.02
Tingling extremities	0.40	0.08 - 1.86	0.24

0.02) lower in women with difficulty concentrating (1/109: 0.9%) than in those without this clinical feature (4/41: 9.8%). Whereas prevalence of high IgG levels was higher, but not statistically significant (P = 0.07), in women suffering from out of control feeling or overwhelmed (5/86: 5.8%) than in those without this clinical characteristic (0/65: 0%). Concerning the association of clinical data and seropositivity to both anti-*T. gondii* IgG and IgM antibodies, bivariate analysis showed no significant associations. DNA of *T. gondii* was found in only one woman who was seropositive to anti-*T. gondii* IgG antibodies. Due to a limited number of cases with high IgG levels, seropositivity to IgM, and positivity to *T. gondii* DNA, no further regression analysis of the association of these laboratory results and the clinical variables was performed.

Discussion

Premenstrual dysphoric disorder is a clinical entity of unclear pathogenesis [18]. This psychiatric disorder is currently affecting up to 8% of women at reproductive age and focuses on psychological symptoms, whereas physical symptoms prevail in premenstrual syndrome [21]. Infection with *T. gondii* leads to a wide parasite spread in the host from the intestine to many organs in the body including the brain [26]. Infection with *T. gondii* has been associated with psychiatric disorders in general [27, 28], and it has been linked to changes in behavior [29, 30]. To our knowledge, the association between *T. gondii* infection and signs and symptoms of premenstrual dysphoric disorder was not assessed previously. Therefore, the present work aimed to determine whether infection with *T. gondii* was associated with clinical characteristics of premenstrual dysphoric disorder in a sample of women in the northern Mexican city of Durango. We observed that women with *T. gondii* IgG antibodies had a similar mean number of signs or symptoms to seronegative women. Nevertheless, multivariate analysis showed that seropositivity to *T. gondii* is associated with specific clinical characteristics of premenstrual dysphoric disorder in particular. Interestingly, IgG seropositivity to *T. gondii* was associated with the clinical manifestation of out of control feeling or overwhelmed. It is not clear why infection with *T. gondii* was associated with this psychological symptom in women with premenstrual dysphoric disorder. We did not find any report in the medical literature about the association of *T. gondii* and the clinical manifestation of out of control feeling or overwhelmed. The subjective sense of being overwhelmed or out of control has been recognized as a diagnostic symptom of premenstrual dysphoric disorder for about 20 years [31]. In a retrospective study of Brazilian college students, researchers found that out of control feeling or overwhelmed were major symptoms of premenstrual dysphoric disorder [32]. Brazil has a high (64.9%) prevalence of *T. gondii* infection among women of childbearing age [33], and our finding raises the question whether *T. gondii* might be linked to these symptoms in Brazilian women with premenstrual dysphoric disorder. In a study in Casablanca, Morocco, researchers found that the subjective sense of being overwhelmed or out of control was present in 55.7% of women with premenstrual dysphoric disorder studied [34]. We may hypothesize that *T. gondii* in brain may lead to psychological symptoms as out of control feeling or overwhelmed in women suffering from premenstrual dysphoric disorder. Infection with *T. gondii* may cause changes in neurotransmitters, i.e., dopamine and serotonin, that could lead to mood and behavioral changes [11, 35]. On the other hand, in the present study, infection with *T. gondii* was negatively associated with difficulty concentrating. This finding suggests a protective effect of *T. gondii* on this clinical characteristic. Nevertheless, this finding may also suggest that *T. gondii* was not an important factor for this symptom in women with premenstrual dysphoric disorder. Difficulty concentrating has been linked to *T. gondii* infection in immunocompetent adult patients suffering from acute toxoplasmic lymphadenitis [10]. Difference in results among the studies might be explained by difference in the characteristics of the patients studied including clinical diagnosis of patients, duration of infection (acute vs. chronic), and gender.

This study has limitations including small sample size of women with premenstrual dysphoric disorder and a low frequency of anti-*T. gondii* IgG, IgM and PCR positivity. In addition, criteria for diagnosis of this disorder have been recently described, and it is unclear whether this disorder requires further characterization. For the above reasons results of the present study should be interpreted with care.

Conclusions

Results of this first study on the association of *T. gondii* infection and clinical characteristics of premenstrual dysphoric disorder suggest that this infection might be linked to some

symptoms of this disorder. We report for the first time the association of *T. gondii* infection and out of control feeling or overwhelmed. Results warrant for further research on the role of *T. gondii* in premenstrual dysphoric disorder.

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Competing Interests

The authors declare that no competing interests exist.

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Toxoplasma gondii exposure and Parkinson's disease: a case–control study

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ABSTRACT

Objectives: To determine the association between *Toxoplasma gondii* infection and Parkinson's disease and to investigate whether *T. gondii* seropositivity is associated with the general characteristics of patients with Parkinson's disease.

Design: Case–control study.

Setting: Cases and controls were enrolled in Durango City, Mexico.

Participants: 65 patients with Parkinson's disease and 195 age- and gender-matched control subjects without Parkinson's disease.

Primary and secondary outcome measures: Serum samples of participants were analysed for anti-*T. gondii* IgG and IgM antibodies by commercially available enzyme-linked immunoassays. Prevalence of *T. gondii* DNA was determined in seropositive subjects using PCR. The association between clinical data and infection was examined by bivariate analysis.

Results: Anti-*T. gondii* IgG antibodies were found in 6/65 cases (9.2%) and in 21/195 controls (10.8%) (OR 0.84; 95% CI 0.32 to 2.18; p=0.81). The frequency of high (>150 IU/mL) antibody levels was similar among cases and controls (p=0.34). None of the anti-*T. gondii* IgG positive cases and four of the anti-*T. gondii* IgG positive controls had anti-*T. gondii* IgM antibodies (p=0.54). The prevalence of *T. gondii* DNA was comparable in seropositive cases and controls (16.7% and 25%, respectively; p=1.0). Seroprevalence of *T. gondii* infection was associated with a young age onset of disease (p=0.03), high Unified Parkinson Disease Rating Scale scores (p=0.04) and depression (p=0.02). Seropositivity to *T. gondii* infection was lower in patients treated with pramipexole than in patients without this treatment (p=0.01). However, none of the associations remained significant after Bonferroni correction.

Conclusions: The results do not support an association between *T. gondii* infection and Parkinson's disease. However, *T. gondii* infection might have an influence on certain symptoms of Parkinson's disease. Further research to elucidate the role of *T. gondii* exposure on Parkinson's disease is warranted.

Strengths and limitations of this study

- This study provides evidence for a better understanding on the association of *Toxoplasma gondii* infection and Parkinson's disease.
- This is the first study that adds molecular detection of *T. gondii* to assess its link with Parkinson's disease.
- Matching by age and sex was performed.
- This study provides clinical characteristics of Parkinson's disease associated with *T. gondii* infection.
- The seroprevalence of *T. gondii* infection was low.

INTRODUCTION

Toxoplasma gondii (*T. gondii*) is an Apicomplexan parasite of medical importance.[1] Infections with *T. gondii* are common and occur worldwide.[2] The main routes of human infection with *T. gondii* include ingestion of water or food contaminated with parasite oocysts shed by cats and consumption of raw or undercooked meat containing parasite tissue cysts.[3] In rare cases, transmission of *T. gondii* may occur by blood transfusion or transplantation.[4] *T. gondii* spreads to a number of organs of the infected host and is able to cross biological barriers and enter into the brain, eye and placenta.[5] Primary infection with *T. gondii* during pregnancy may lead to infection of the fetus.[6] The clinical spectrum of *T. gondii* infection varies from asymptomatic to severe disease with lymphadenopathy, chorioretinitis and meningoencephalitis.[3 6 7]

Infection with *T. gondii* has been linked to a number of neuropsychiatric diseases including schizophrenia, Parkinson's disease and Alzheimer's disease, and the

neurobiological data of this link have recently been reviewed.[8] The aetiology of Parkinson's disease is largely unknown; however, progressive impairment of voluntary motor control—which is a clinical feature of this disease—is caused by a loss of midbrain substantia nigra dopamine neurons.[9] Tissue cysts of *T. gondii* may be found in all brain areas,[10] and *T. gondii* may lead to neurological damage.[11] It therefore raises the question whether infection with *T. gondii* may lead to Parkinson's disease. On the other hand, infection with *T. gondii* may increase the production of dopamine in the brain.[12] Therefore, it also raises the question whether Parkinson's disease could be negatively associated with infection with *T. gondii*. However, the potential link of *T. gondii* infection and Parkinson's disease has been poorly investigated, and conflicting results about the association of *T. gondii* exposure and Parkinson's disease have been reported. Miman *et al*[13] found a significantly higher rate of anti-*T. gondii* IgG antibodies in patients with Parkinson's disease than in controls. In contrast, Celik *et al*[14 15] found similar seropositivity rates to *T. gondii* in 50 patients with idiopathic Parkinson's disease and 50 healthy volunteers. In addition, Oskouei *et al*[16] found similar prevalences of anti-*T. gondii* IgG antibodies in 75 patients with Parkinson's disease and 75 controls. Given these conflicting results, we assessed the association of *T. gondii* infection and Parkinson's disease in a cohort of patients attending public hospitals in Durango City, Mexico. In addition, we investigated the association of *T. gondii* seropositivity and the sociodemographic and clinical characteristics of patients with Parkinson's disease.

MATERIALS AND METHODS

Patients with Parkinson's disease and controls

We performed a case–control study of 65 patients with Parkinson's disease (cases) and 195 control subjects. Diagnosis of Parkinson's disease was made using the UK Parkinson's Disease Society brain bank clinical diagnostic criteria.[17] Patients were enrolled in the departments of neurology at two public hospitals: the Hospital 'Santiago Ramón y Cajal' of the Institute of Security and Social Services for the State Workers, and the Hospital '450' of the Secretary of Health in Durango City, Mexico. Serum samples were obtained from January to December 2014. Inclusion criteria for the cases were patients with Parkinson's disease of either sex who voluntarily accepted to participate in the study. Exclusion criteria for the cases were presence of renal or liver diseases, gout, alcoholism, history of cerebrovascular disease or other neurological diseases, and use of acetylsalicylic acid or allopurinol. Cases were aged 39–95 years (mean 69.08±11.39 years) and included 30 men and 35 women. We used a convenience sampling to enrol cases. Inclusion criteria for controls were subjects from the general population of the same city without neurological disease, matched with cases by age and sex. We included three controls per case. Controls were aged 38–91 years (mean 68.56±10.08 years) and included 90 men and 105 women. There was no difference in age between cases and controls (p=0.85).

Sociodemographic and clinical data of cases

We obtained the sociodemographic and clinical data of the patients with Parkinson's disease through face-to-face neurological consultations and with the aid of a questionnaire. Since the correlation of *T. gondii* infection with clinical features of Parkinson's disease is largely unknown, we explored the association between *T. gondii* seropositivity and a number of clinical characteristics directly or indirectly associated with Parkinson's disease. Sociodemographic data obtained included age and sex. Clinical data included Hoehn and Yahr stages,[18] Unified Parkinson Disease Rating Scale scores, age at onset of Parkinson's disease, duration of disease, presence of tremor or rigidity at disease onset, most affected body side, familial history of Parkinson's disease, presence of hyposmia, syncope, paraesthesias, dementia, impairments of memory and vision, depression, anxiety, sialorrhoea, constipation, weight loss, sleep disorders, erectile dysfunction and orthostatic hypotension. In addition, information about the presence of obesity, dyslipidaemia, diabetes mellitus, arterial hypertension, smoking, diarrhoea, nausea and/or vomiting was obtained from each patient. Antiparkinsonian medication was also registered and included the use of levodopa, carbidopa, pramipexole, trihexyphenidyl, biperiden, amantadine, rasagiline, selegiline, azilect, rotigotine and bromocriptine. The occurrence of dyskinesia, urinary incontinence and motor fluctuations (ie, end-of-dose wearing-off, unpredictable off, delay on and no on) related to treatment was also recorded.

Detection of anti-*T. gondii* antibodies

Anti-*T. gondii* IgG antibodies were detected in the serum of participants using the commercially available enzyme immunoassay *Toxoplasma* IgG kit (Diagnostic Automation, Woodland Hills, California, USA). This test determines the presence and also the levels of IgG antibodies. A cut-off of 8 IU/mL of specific anti-*T. gondii* IgG antibody was used. All serum samples positive for anti-*T. gondii* IgG antibodies were further analysed for anti-*T. gondii* IgM antibodies by the commercially available enzyme immunoassay *Toxoplasma* IgM kit (Diagnostic Automation). All tests were performed following the manufacturer's instructions.

Detection of *T. gondii* DNA by PCR

Whole blood samples of cases and controls with anti-*T. gondii* IgG antibodies were further examined to detect *T. gondii* DNA by nested PCR. Whole blood extraction of DNA followed the protocol described by Iranpour and Esmailizadeh (http://www.protocol-online.org/prot/Protocols/Rapid-Extraction-of-High-Quality-DNA-from-Whole-Blood-Stored-at-4-C-for-Long-Period-4175.html). A PCR protocol with two pairs of primers directed

against the B1 gene of *T. gondii* was used, as previously described.[19] The amplified PCR products were detected using gel electrophoresis, stained with ethidium bromide and visualised under ultraviolet light.

Statistical analysis

We used the software Microsoft Excel 2010, Epi Info V.7 (Centers for Disease Control and Prevention: http://wwwn.cdc.gov/epiinfo/) and SPSS V.15.0 (SPSS, Chicago, Illinois, USA) to analyse the results. For calculation of the sample size we used a 95% confidence level, power of 80%, 1:3 proportion of cases and controls and a reference seroprevalence of 12.0%[20] as the expected frequency of exposure in controls. The result of the sample size calculation was 60 cases and 179 controls. To avoid bias, we excluded subjects with missing clinical data. Age values among the groups were compared with the paired Student's t-test. The Fisher exact test was used to evaluate the association between seropositivity to *T. gondii* and the characteristics of the patients. ORs and 95% CIs were calculated and a p value <0.05 was considered statistically significant. Bonferroni correction was applied for adjustment of multiple testing.

RESULTS

Anti-*T. gondii* IgG antibodies were found in 6/65 cases (9.2%) and in 21/195 controls (10.8%) (OR 0.84; 95% CI 0.32 to 2.18; p=0.81). Of the six anti-*T. gondii* IgG positive cases, five (83.3%) had anti-*T. gondii* IgG antibody levels >150 IU/mL and one (16.7%) 12 IU/mL. In contrast, of the 21 anti-*T. gondii* IgG positive controls, 11 (52.4%) had anti-*T. gondii* IgG antibody levels >150 IU/mL, one (4.8%) between 100 to 150 IU/mL and 9 (42.8%) between 8 and 99 IU/mL. The frequency of high (>150 IU/mL) antibody levels was similar among cases and controls (p=0.34). None of the six anti-*T. gondii* IgG positive cases had anti-*T. gondii* IgM antibodies whereas four (19.0%) of the 21 anti-*T. gondii* IgG positive controls had anti-*T. gondii* IgM antibodies. There was no difference in the rate of IgM seropositivity among cases and controls (p=0.54). Anti-*T. gondii* IgG antibodies were detected in four (11.4%) of 35 female cases and in seven (6.7%) of 105 female controls (OR 1.80; 95% CI 0.49 to 6.58; p=0.46), whereas anti-*T. gondii* IgG antibodies were detected in two (6.7%) of 30 male cases and in 14 (15.6%) of 90 male controls (OR 0.38; 95% CI 0.08 to 1.81; p=0.35). The frequency of high (>150 IU/mL) anti-*T. gondii* IgG antibody levels was similar in male and female cases (2/30 (6.7%) and 3/35 (8.6%), respectively, p=1.00). Seroprevalence of *T. gondii* infection was similar among cases and controls of several age groups (table 1). One (16.7%) of the six cases seropositive to *T. gondii* IgG antibodies was positive for *T. gondii* DNA by PCR. We were able to test 20 of 21 controls seropositive to *T. gondii* IgG antibodies. Five (25%) of these 20 controls were positive for *T. gondii* DNA by PCR. The prevalence of *T. gondii* DNA was similar in cases and controls (p=1.0).

With respect to clinical characteristics of patients, seroprevalence of *T. gondii* infection was higher in patients with an onset of Parkinson's disease at a young age (≤40 years) than in those with a disease onset at older ages (p=0.03). Table 2 shows a selection of clinical characteristics of patients with Parkinson's disease and their correlation with *T. gondii* seropositivity. Seroprevalence of infection with *T. gondii* was also higher in patients with higher Unified Parkinson Disease Rating Scale scores (88–136) than in those with lower scores (p=0.04). Seropositivity to *T. gondii* was observed in six (17.1%) of 35 patients suffering from depression but in none of 30 patients without depression (p=0.02). Other clinical characteristics of patients including Hoehn and Yahr stages, duration of disease, presence of tremor or rigidity at disease onset, most affected body side, familial history of Parkinson's disease, presence of hyposmia, syncope, paraesthesias, dementia, impairments of memory and vision, anxiety, sialorrhoea, constipation, weight loss, sleep disorders, erectile dysfunction, and orthostatic hypotension did not show an association with *T. gondii* seropositivity. In addition, *T. gondii* exposure was not associated with the presence of obesity, dyslipidaemia, diabetes mellitus, arterial hypertension, smoking, diarrhoea, nausea and/or vomiting in the patients. Seropositivity to *T. gondii* infection was significantly (p=0.01) lower in patients receiving pramipexole than in patients not treated with this drug (table 2). Seroprevalence of infection was similar in patients regardless of the use of other antiparkinsonian medications including levodopa, carbidopa, trihexyphenidyl, biperiden, amantadine, rasagiline, selegiline, azilect, rotigotine and bromocriptine. The presence of

Table 1 Comparison of IgG seropositivity rates in cases and controls according to age groups

	Cases			Controls			
	Subjects tested	Seropositive		Subjects tested	Seropositive		
	N	N	%	N	N	%	p Value
Age groups							
≤40 years	2	1	50	6	0	0	0.25
41–60 years	12	1	8.3	22	1	4.5	1.00
61–80 years	41	4	9.8	144	17	11.8	1.00
>80 years	10	0	0	23	3	13	0.53

Table 2 Bivariate analysis of clinical data and infection with *Toxoplasma gondii* in patients with Parkinson's disease

Characteristic	Subjects tested N	Prevalence of *T. gondii* infection N	%	p Value
Age at Parkinson onset				
≤40 years	4	2	50	0.03
>40 years	61	4	6.6	
Duration of disease				
≤10 years	57	5	8.8	0.56
>10 years	8	1	12.5	
Tremorigenic type				
Yes	49	5	10.2	1.00
No	16	1	6.3	
Rigid type				
Yes	25	3	12	0.66
No	40	3	7.5	
Hoehn and Yahr stages				
0	5	0	0	0.59
1	17	3	17.6	
2	14	1	7.1	
3	20	1	5	
4	5	1	20	
5	4	0	0	
Unified Parkinson disease rating scores				
0–87	55	3	5.5	0.04
88–136	10	3	30	
Constipation				
Yes	29	4	13.8	0.39
No	36	2	5.6	
Syncope				
Yes	6	1	16.7	0.45
No	59	5	8.5	
Paraesthesias				
Yes	12	3	25	0.07
No	53	3	5.7	
Weight loss				
Yes	27	4	14.8	0.22
No	38	2	5.3	
Dementia				
Yes	23	3	13	0.65
No	42	3	7.1	
Depression				
Yes	35	6	17.1	0.02
No	30	0	0	
Anxiety				
Yes	30	4	13.3	0.40
No	35	2	5.7	
Vision impairment				
Yes	22	3	13.6	0.39
No	43	3	7	
Dyskinesia				
Yes	21	3	14.3	0.37
No	44	3	6.8	
Use of pramipexole				
Yes	43	1	2.3	0.01
No	22	5	22.7	

dyskinesia, urinary incontinence and motor fluctuations (end-of-dose wearing-off, unpredictable off, delay on and no on) did not correlate with *T. gondii* infection. None of the associations between clinical data and *T. gondii* seropositivity remained significant after Bonferroni correction.

DISCUSSION

T. gondii is an intracellular parasite and can persist in neurons, modifying their function and structure.[21] Cysts of *T. gondii* can be found throughout the brain,[10] and this parasite alters dopamine metabolism.[21] Thus, it raises the question whether infection with *T. gondii* has any link with a dopamine-related neurological disease. There is controversy concerning the association of *T. gondii* infection and Parkinson's disease. The number of reports about this association is very small. We therefore sought to determine the association between *T. gondii* seropositivity and patients with Parkinson's disease in the northern Mexican city of Durango. This age- and gender-matched case–control seroprevalence study showed similar frequencies of *T. gondii* infection in cases and controls. Similarly, we did not find differences in the frequency of high levels of anti-*T. gondii* IgG antibodies, IgM seropositivity rates and prevalence of *T. gondii* DNA among cases and controls. The 9.2% seroprevalence found in patients with Parkinson's disease is comparable to the 12% seroprevalence of *T. gondii* infection reported in elderly people[20] and 13.3% in patients with liver disease[22] in the same Durango City. In contrast, the seroprevalence found in patients with Parkinson's disease is lower than seroprevalences reported in other population groups in Durango City including 15.4% in female sex workers,[23] 20% in schizophrenic patients[24] and 21.1% in inmates[25] and waste pickers.[26] Therefore, the results of our study do not support an association between *T. gondii* infection and Parkinson's disease. The lack of association between *T. gondii* infection and the presence of Parkinson's disease is consistent with similar results reported by Celik *et al*[14 15] and Oskouei *et al*.[16]

In contrast, our results conflict with those reported by Miman *et al*[13] who found a significantly higher seroprevalence of anti-*T. gondii* IgG antibodies in patients with Parkinson's disease than in controls. Other studies have also linked toxoplasmosis with Parkinson's disease. For instance, in 1992 Noël *et al*[27] reported hemichorea and parkinsonism in two AIDS patients with cerebral toxoplasmosis. Basal ganglia, which are involved in the control of voluntary motor movements, can be affected in cerebral toxoplasmosis, as reported in patients with AIDS,[28–30] a patient with acute myeloid leukaemia undergoing two allogenic stem cell transplantations,[31] an immunocompromised female renal transplant recipient[32] and a non-immunocompromised pregnant woman.[33] Improvement of parkinsonism in an AIDS patient with cerebral toxoplasmosis was achieved after anti-*T. gondii* and antiretroviral therapies.[34] Infection with *T. gondii* has been associated with elevated levels of dopamine within dopaminergic cells,[12] whereas an important feature of Parkinson's disease is the loss of dopamine-producing neurons.[35] However, the interaction of *T. gondii* and neurons in patients with Parkinson's disease is largely unknown. It raises the question whether dopamine production during infection with *T. gondii* is too low to compensate for the deficit of dopamine and to induce a clinical improvement in patients with Parkinson's disease. Further research to elucidate the role of dopamine produced during *T. gondii* infection on neurons of patients with Parkinson's disease is needed.

Interestingly, the frequency of *T. gondii* infection was higher in patients with onset of Parkinson's disease at a young age than in those with a disease onset at older ages. It is not clear why *T. gondii* infection was associated with a young onset of Parkinson's disease. This young onset of disease is less common than middle and late onsets, and patients with young onset have a long survival and suffer from depression more frequently than patients with older onset of disease.[36] Remarkably, we found that seropositivity to *T. gondii* was associated with depression in the patients with Parkinson's disease studied. To the best of our knowledge, this is the first report of an association between *T. gondii* exposure and depression in patients with Parkinson's disease. Infection with *T. gondii* has been linked to depression in other population groups, such as women veterans[37] and pregnant women.[38] However, other studies including a meta-analysis of 50 studies of psychiatric patients and healthy controls,[39] a cross-sectional internet study on a non-clinical population of 5535 subjects[40] and the third National Health and Nutrition Survey in the USA[41] have not found a correlation between *T. gondii* infection and depression

Of note, seroprevalence of *T. gondii* infection correlated with high Unified Parkinson Disease Rating Scale scores. In a search for this association in the medical literature, no reports were found. This association suggests that *T. gondii* infection might have an influence on clinical characteristics of patients with Parkinson's disease. It is possible that *T. gondii* does not associate per se with the presence of Parkinson's disease because of the opposite relations with dopamine production—that is, *T. gondii* infection induces an increase in dopamine production whereas Parkinson's disease is related to a decrease in dopamine production. However, infection with *T. gondii* might be involved in the appearance of symptoms found in patients with Parkinson's disease such as depression. Further research on the influence of *T. gondii* infection on signs and symptoms of Parkinson's disease should be conducted.

We also observed that seropositivity to *T. gondii* infection was significantly lower in patients treated with pramipexole than in those not receiving this treatment. This finding suggests a protective effect of pramipexole for *T. gondii* infection. It is not clear why pramipexole users had a low frequency of *T. gondii* infection. No anti-*T. gondii* activity of pramipexole has been reported. Further research to elucidate the negative association of pramipexole with *T. gondii* infection is needed.

This study has limitations. The sample size was small. Further studies with larger sample sizes should be conducted. The low number of cases seropositive for

T. gondii did not allow us to perform multivariate analysis to determine the association between patient characteristics and seropositivity to *T. gondii*. In addition, the associations between clinical data and *T. gondii* seropositivity found in this study should be interpreted with care, since the statistical power of comparisons was low (<0.80) and no associations remained statistically significant after Bonferroni correction.

CONCLUSIONS

The results obtained in a cohort of patients in Durango, Mexico do not support an association between *T. gondii* infection and Parkinson's disease. However, the results suggest that *T. gondii* infection might influence the symptoms of Parkinson's disease. Further research to elucidate the role of *T. gondii* exposure on the clinical characteristics of Parkinson's disease is therefore needed.

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Contributors CA-E designed the study protocol, performed the laboratory tests and data analysis and wrote the manuscript. EMM-H, JMS-P, LAR-C and AAS-C obtained the blood samples and clinical dat, and performed the data analysis. JH-T, OA-C, LFS-A, FXC-J and OL performed the data analysis and wrote the manuscript. All authors read and approved the final version of the manuscript.

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ORIGINAL ARTICLE

Lack of Serological and Molecular Association between *Toxoplasma Gondii* Exposure and Obesity: A Case-Control Study

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ABSTRACT

The association between *T. gondii* infection and obesity has been scantly studied. Through an age-, and gender-matched case-control study, we determined the association of *T. gondii* infection and obesity using serological and molecular methods. Cases included 203 persons with obesity, and controls included 203 persons without obesity. Participants were tested for the presence of anti-*Toxoplasma* IgG antibodies using an enzyme-linked immunoassay (EIA). IgG seropositive individuals were further tested for the presence of anti-*T. gondii* IgM antibodies using an EIA, and *T. gondii* DNA by polymerase chain reaction (PCR). Anti-*T. gondii* IgG antibodies were found in 16 (7.9%) of the 203 cases and in 18 (8.9%) of the 203 controls (OR=0.87; 95% CI: 0.43-1.77; *P*=0.72). One (6.3%) of the 16 anti-*T. gondii* IgG seropositive cases and 6 (33.3%) of the 18 anti-*T. gondii* IgG seropositive controls were positive for IgM (*P*=0.09). Mean body mass index (35.5 ± 4.5) in *T. gondii* seropositive cases was similar (*P*=0.57) to that (36.1 ± 4.5) found in *T. gondii* seronegative cases. Stratification by obesity classes (I, II, and III) did not reveal differences (*P*>0.05) in seroprevalences (7.8%, 7.9%, and 8.1%, respectively) or high (>150 IU/ml) IgG antibody levels (3.3%, 3.9%, and 2.7%, respectively). PCR was positive in 5 (31.3%) of 16 cases, and in 5 (27.8%) of 18 controls examined (*P*=1.0). We found no serological or molecular evidence of an association between *T. gondii* infection and obesity in people attending a public health center in the northern Mexican city of Durango. (*Int J Biomedi Sci* 2017; 13 (2): 74-78)

Keywords: *Toxoplasma gondii*; seroprevalence; obesity; case-control study

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INTRODUCTION

The coccidian parasite *Toxoplasma gondii* (*T. gondii*) is a common pathogen with worldwide distribution (1). This parasite is usually transmitted to humans by ingestion of food or water contaminated with oocysts shed by cats, and ingestion of raw or undercooked meat containing tissue cysts (2, 3). In addition, primary infection with *T. gondii* in pregnant women may lead to vertical transmission with risk for congenital disease (4). Infection with *T. gondii* is usually asymptomatic, however, this infection may lead to disease of the central nervous system, eyes and lymph nodes (5, 6). In immunocompromised individuals, a reactivation of *T. gondii* infection may result in a severe and life-threatening disease with involvement of the central nervous system (2, 7). Infection with *T. gondii* has been associated with changes in behavior in humans and animals (8, 9). An increase in dopamine production induced by *T. gondii* may contribute to behavioral changes (8). Several psychiatric disorders have been linked to infection with *T. gondii* including schizophrenia (10), bipolar disorder, and obsessive-compulsive disorder (11).

Obesity is a major health problem, and its prevalence is high in many parts of the world (12). In Mexico, more than 50% of adults have overweigh and obesity (13). Obesity and overweight have been linked to important causes of mortality in Mexico including coronary heart disease, type-2 diabetes mellitus, cancer, and stroke (13). Infection with *T. gondii* might be associated with obesity because this infection is usually acquired by food. Obese people may eat in a higher quantity than non-obese people; and therefore, this increase in eating might increase the risk of consuming food likely contaminated with *T. gondii*. It is possible that consumption of a double portion of meat (a well-known source of *T. gondii*) might increase two-fold the risk for acquiring infection. It is also possible that drinking untreated water or consuming unwashed raw vegetables or fruits in high quantities might also increase the risk for *T. gondii* infection. The association of *T. gondii* infection and obesity has been scantly studied. Reeves and coworkers (14) found an association between positive serology to *T. gondii* and obesity in psychiatrically healthy adults. Rubicz and coworkers (15) found a 9% seroprevalence of *T. gondii* infection in Mexican Americans from San Antonio, Texas that suffered from high rates of obesity and type-2 diabetes. In contrast, in a multinational epidemiological study of individuals from Iceland, Sweden and Estonia, no association of anti-*T. gondii* IgG antibodies and body mass index was found (16). However, in a recent study in Germany a body mass index ≥30 was an independent risk factor for IgG seropositivity to *T. gondii* (17). The present study therefore aimed to determine whether *T. gondii* infection is associated with obesity in adults attending a public clinic of family medicine in Durango City, Mexico. Determining this association may help for an optimal planning of preventive measures against *T. gondii* infection.

METHODS

Selection and description of participants

Through an age- and gender-matched case control study design, we studied 203 individuals with obesity and 203 individuals without obesity attended in a public clinic of familiar medicine in Durango City, Mexico. This study was performed from June 2015 to August 2016. Inclusion criteria for enrollment of cases were: 1) individuals with obesity attending a public primary health care center (Clinic of Family Medicine, Institute of Security and Social Services of State Workers) in Durango City, Mexico; 2) aged 18 years and older; and 3) who accepted to participate in the study. Socioeconomic status and occupation were not restrictive criteria for enrollment.

Obesity was defined as a body mass index ≥30; and classified in class I, class II, and class III when body mass indexes were 30-34.9, 35.0-39.9, and ≥40.0, respectively (13). Control subjects were matched with cases for age and gender. Cases included 42 (20.7%) males and 161 (79.3%) females, and their mean age was 51.4 ± 11.6 (range 22-83) years old. Controls were randomly selected. Inclusion criteria for enrollment of controls subjects were: 1) individuals without obesity attending the same public primary health care center where cases were selected; 2) aged 18 years and older; and 3) who accepted to participate in the study. Controls included 42 (20.7%) males and 161 (79.3%) females. Mean age in control subjects were 51.5 ± 11.5 (range 20-80) years old. No statistically significant difference (P=0.89) in age between cases and controls was found.

Technical information

Sera from cases and controls were obtained and kept frozen at -20°C until analyzed. Anti-*T. gondii* IgG antibodies were detected in sera using the commercially available enzyme immunoassay (EIA) kit "*Toxoplasma* IgG" (Diagnostic Automation/Cortez Diagnostics Inc., Woodland Hills, CA, USA). Anti-*T. gondii* IgG antibody levels were expressed as International Units (IU)/ml. We used

a cut-off of 8 IU/ml for seropositivity. All serum samples positive for anti-*T. gondii* IgG antibodies were further analyzed for anti-*T. gondii* IgM antibodies using the commercially available EIA "*Toxoplasma* IgM" kit (Diagnostic Automation/Cortez Diagnostics Inc.). Both IgG and IgM EIAs were performed following the manufacturer's instructions.

Cases and controls seropositive for *Toxoplasma*-specific IgG antibodies by EIA were further analyzed to detect DNA of *T. gondii* by nested-polymerase chain reaction (PCR). DNA was extracted from whole blood samples of cases and controls according to a protocol described by Iranpour and Esmailizadeh [http://www.protocol-online.org/prot/Protocols/Rapid-Extraction-of-High-Quality-DNA-from-Whole-Blood-Stored-at-4-C-for-Long-Period-4175.html]. PCR amplification was performed following the PCR protocol described by Roth *et al* (18). Primers directed against the B1 gene of *T. gondii* were used. PCR amplified material was analyzed by agarose gel electrophoresis, stained with ethidium bromide, and visualized by ultraviolet illumination.

Statistics

Data was analyzed using the software Epi Info 7 and SPSS 15.0 (SPSS Inc. Chicago, Illinois). We calculated the sample size using the following values: a 95% confidence level, a power of 80%, a 1:1 proportion of cases and controls, and a reference seroprevalence of 6.1% (19) as the expected frequency of exposure in controls, and an odds ratio of 2.8. Thus, a sample size of 195 cases and 195 controls was obtained. Age among cases and controls was compared with the student's *t* test. The association of *T. gondii* infection and obesity was analyzed with the two-tailed Pearson's chi-squared test. We calculated the odds ratio (OR) and 95% confidence interval (CI), and statistical significance was set at a P value < 0.05.

Ethics aspects

The ethics committee of the Institute of Security and Social Services of State Workers in Durango City, Mexico approved this study. Participation in the study was voluntary, and a written informed consent was obtained from each participant.

RESULTS

Anti-*T. gondii* IgG antibodies were found in 16 (7.9%) of the 203 cases and in 18 (8.9%) of the 203 controls. The seroprevalence of *T. gondii* infection in cases was similar to the one in controls (OR=0.87; 95% CI: 0.43-1.77; P=0.72). Of the 16 anti-*T. gondii* IgG positive cases, 7 (43.8%) had IgG levels higher than 150 IU/ml, one (6.3%) between 100-150 IU/ml, and 8 (50.0%) between 8 to 99 IU/ml. Whereas, of the 18 anti-*T. gondii* IgG positive controls, 13 (72.2%) had IgG levels higher than 150 IU/ml, and 5 (27.8%) between 8 to 99 IU/ml. The frequency of high (>150 IU/ml) anti-*T. gondii* IgG levels in cases was similar to the one in controls (OR=0.29; 95% CI: 0.07-1.24; P=0.18). One (6.3%) of the 16 anti-*T. gondii* IgG seropositive cases was positive to anti-*T. gondii* IgM antibodies by EIA. In contrast, 6 (33.3%) of the 18 anti-*T. gondii* IgG seropositive controls were positive to IgM by EIA. No difference in the frequencies of anti-*T. gondii* IgM antibodies among cases and controls was found (P=0.09).

Mean body mass index in *T. gondii* seropositive cases (35.5 ± 4.5) was similar (P=0.57) to that (36.1 ± 4.5) found in *T. gondii* seronegative cases. Stratification by obesity classes I, II, and III did not show differences (P>0.05) in seroprevalences (7.8%, 7.9%, and 8.1%, respectively) or frequency of high IgG antibody levels (3.3%, 3.9%, and 2.7%, respectively).

With respect to detection of *T. gondii* DNA in whole blood of anti-*T. gondii* IgG positive participants, PCR was positive in 5 (31.3%) of 16 cases and in 5 (27.8%) of 18 controls examined. No statistically significant difference in the frequencies of *T. gondii* DNA positivity among cases and controls was found (P=1.0). Stratification by age and gender groups did not show differences (P>0.05) in seroprevalences among cases and controls (Table 1). *T. gondii* DNA was found in three cases with >150 IU/ml of IgG antibodies and in two cases with <100 IU/ml of IgG antibodies. All 5 cases with *T. gondii* DNA were negative to anti-*T. gondii* IgM antibodies. *T. gondii* DNA was found in three cases with obesity class I, in one case with obesity class II, and in one case with obesity class III.

DISCUSSION

Very little is known about the association of *T. gondii* infection and obesity. Results of a few studies about this association have shown conflicting results (14-17). Positive association between seroprevalence of *T. gondii* infection and obesity has been found in adults in Germany (14, 17). In contrast, a low (9%) seroprevalence of *T. gondii* infection in Mexican Americans from San Antonio, Texas that suffered from high rates of obesity and type-2 diabetes was found (15). In addition, no association of seroprevalence of *T. gondii* infection and body mass index was

Table 1. Correlation of *T. gondii* seropositivity and demographic variables in cases and controls

Variable	Cases			Controls			*P.* value
	No. of subjects tested	Seropositive to T. gondii		No. of subjects tested	Seropositive to T. gondii		
		No.	%		No.	%	
Ages (years)							
30 or less	12	0	0.0	10	1	10.0	0.45
31-50	76	5	6.6	73	7	9.6	0.55
>50	115	11	9.6	120	10	8.3	0.82
Gender							
Female	161	12	7.5	161	9	5.6	0.49
Male	42	4	9.5	42	9	21.4	0.13

found in a multinational epidemiological study in Iceland, Sweden and Estonia, (16). Therefore, we sought to determine whether *T. gondii* infection is associated with obesity in adults attending a public clinic of family medicine in the northern Mexican city of Durango. For this purpose, we assessed not only the prevalence of anti-*T. gondii* IgG antibodies but also the IgG levels, anti-*T. gondii* IgM seropositivity, and detection of *T. gondii* DNA. Results of the present study indicate that anti-*T gondii* IgG and IgM seropositivity rates, IgG levels, and frequency of *T. gondii* DNA in obese people are similar to those observed in age- and gender-matched control subjects without obesity attended in the same clinic of family medicine. Therefore, our results based on serological and molecular methods do not support an association between obesity and *T. gondii* infection. Results of the present study agree with the lack of association between body mass index and *T. gondii* IgG seroprevalence found in a multinational epidemiological study of individuals from Iceland, Sweden and Estonia (16), and with the low (9%) seroprevalence of *T. gondii* infection found in Mexican Americans that suffered from high rates of obesity and type-2 diabetes reported by Rubicz and coworkers (15). In contrast, our results conflict with those reported in two German studies (14, 17). A positive serology to *T. gondii* associated with obesity in psychiatrically healthy adults in Germany was reported by Reeves and coworkers (14). Furthermore, a body mass index ≥30 was an independent risk factor for IgG seropositivity to *T. gondii* in a nationwide representative cross-sectional study in Germany (17). It is not clear why the association of *T. gondii* infection and obesity was found in populations in Germany but not in obese people in the present study. It is likely that differences in the characteristics of the populations and study designs among the studies might explain the differences in the association. In the study of Reeves and coworkers (14), the association between *T. gondii* infection and obesity was observed in subjects 60 years and older but not in subjects younger than 60 years. Stratification by age groups in our study (<30, 31-50, and >50 years) did not show an association of infection and obesity. In addition, we used an age- and gender-matched case-control study design whereas Reeves and coworkers performed adjustment by age but not by gender. The number of obese participants in the study by Reeves and coworkers was 74 (14), whereas we studied 203 obese participants. On the other hand, in the study of Wilking and coworkers (17) who reported an association of *T. gondii* seropositivity and obesity, researchers studied a large number (1,023) of obese participants, but the study design was cross-sectional, and no adjustment or stratification by age for *T. gondii* seropositivity was performed. In addition, Wilking and coworkers (17) used an automatic enzyme-linked fluorescence assay for detection of anti-*T. gondii* IgG antibodies whereas we used a manual enzyme-linked immunosorbent assay. We are not aware of a previous study about the association of *T. gondii* infection and obesity using molecular methods. However, in the present study using *T. gondii* PCR, no association between obesity and *T. gondii* DNA was found. The lack of association between obesity and *T. gondii* was unexpected since obese people may eat more than non-obese people; and therefore, an increase in eating might increase the risk of consuming food likely contaminated with *T. gondii*.

The limitations of the present study include the investigation of a relatively small cohort of obese people attending a single public health center. The socioeconomic sta-

tus of people attending the participating health center are mostly medium, and it is not clear whether the association of obesity and *T. gondii* infection might occur in people of low or high socioeconomic status.

CONCLUSIONS

We conclude that there is not serological or molecular evidence of an association between *T. gondii* infection and obesity in people attended in a public family medicine health center in the northern Mexican city of Durango. Further research to elucidate the role of *T. gondii* in obesity is needed.

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ABBREVIATIONS

CI	Confidence interval
EIA	Enzyme immunoassay
IU	International units
OR	Odds ratio
PCR	Polymerase chain reaction

CONFLICT OF INTEREST

The authors declare that no conflict of interest exists.

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Original article

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LACK OF ASSOCIATION BETWEEN CYTOMEGALOVIRUS INFECTION AND HYPERTENSIVE DISORDERS IN PREGNANCY: A CASE-CONTROL STUDY IN DURANGO, MEXICO

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It is not clear whether infection with cytomegalovirus (CMV) is associated with hypertensive disorders in pregnant women. Through a case-control study design, 146 women suffering from hypertensive disorders in pregnancy (cases) and 146 age-matched normotensive pregnant women (controls) were examined for the presence of anti-CMV IgG and IgM antibodies with enzyme-linked immunoassays. IgM seropositive samples were further assayed by enzyme-linked fluorescent assay (ELFA).

Anti-CMV IgG antibodies were found in 138 (94.5%) controls and in 136 (93.2%) cases (odds ratio [OR] = 0.78; 95% confidence interval [CI]: 0.30–2.05; $P = 0.62$). High (>18 IU/ml) levels of anti-CMV IgG antibodies were found in 37.7% of the 138 seropositive controls and in 34.6% of the 136 seropositive cases (OR = 0.87; 95% CI: 0.53–1.43; $P = 0.59$). Anti-CMV IgM antibodies were found in 1 (0.7%) of the controls but in none of the cases using ELFA ($P = 1.0$). Seropositivity to CMV was not associated with a previous preeclampsia and was similar among cases regardless their mean systolic and diastolic blood pressures, and mean arterial blood pressure.

No serological evidence of an association between CMV infection and hypertensive disorders of pregnancy was found. Further research to elucidate the role of CMV in hypertensive disorders in pregnancy should be conducted.

Keywords: cytomegalovirus, seroprevalence, preeclampsia, HELLP syndrome, eclampsia, infection, epidemiology

Introduction

Cytomegalovirus (CMV) is a DNA virus of the Herpesviridae family and is widely distributed around the world [1]. Major routes of CMV infection are person-to-person contact [2] and blood transfusion [3]. Infections with CMV are persistent [4], and their reactivations contribute for shedding of infectious virus [2, 5]. Immunocompromised individuals infected with CMV may develop a severe disease including encephalitis, pneumonia, retinitis, and hepatitis [6]. Furthermore, CMV is an important pathogen leading to congenital infections [7–9]. The clinical spectrum of congenital infections varies from asymptomatic [10] to severe disease including mental retardation, cerebral palsy, hearing loss, and neurodevelopmental delay [9, 11]. Infections with CMV may occur in placenta [12–14]. In addition, inflammation and edema in placenta induced by CMV infections have been observed in cases of preeclampsia [13, 14]. Preeclampsia and other hypertensive disorders in pregnancy are major health problems leading to maternal and perinatal morbidity and mortality [15–17]. Worldwide estimates indicate that about 8.5 mil-

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lion pregnant women suffer from preeclampsia every year [18], and 4.6% and 1.4% of all deliveries are associated with preeclampsia and eclampsia, respectively [19]. The role of CMV in hypertension disorders in pregnancy is controversial. Several serological studies have failed to demonstrate a correlation of CMV infection and hypertensive disease in pregnancy [20–22]. In contrast, several serological studies have found an association of CMV infection with preeclampsia [23–25]. Therefore, we sought to determine the association of CMV infection and hypertensive disorders in pregnant women in Durango City, Mexico.

Materials and methods

Study design and study populations

We performed a case-control study to assess the association of CMV infection with hypertensive disorders in pregnancy using serum samples from a recent *Toxoplasma gondii* study in women in Durango City, Mexico [26]. Serum samples were obtained in a public hospital (General Hospital) from the Secretary of Health from November 2011 to September 2013. Cases and controls were matched by gender, age, attending hospital, and residence place. A 1:1 ratio for matching was used.

Women suffering from hypertensive disorders in pregnancy

Inclusion criteria for the group of cases were: 1) pregnant women at their 24–42 weeks of pregnancy suffering hypertensive disorders and proteinuria attended in the Department of Gynecology and Obstetrics of the General Hospital in Durango City, Mexico; and 2) who agreed voluntary participation in the study. Hypertensive disorders during pregnancy were mild preeclampsia, severe preeclampsia, eclampsia, and HELLP syndrome. Mild preeclampsia was defined as blood pressure ≥140/90 mmHg on 2 occasions, at least 6 h apart, and proteinuria of ≥300 mg/24 h. Severe preeclampsia was considered as blood pressure ≥160/110 mmHg on 2 occasions, at least 6 h apart, and proteinuria of ≥5 g/24 h. Eclampsia was diagnosed when hypertension, proteinuria, and seizures in a patient were found. HELLP syndrome was defined as hypertension, proteinuria and presence of hemolytic anemia, elevated liver enzymes, and low platelet count. All eligible women attended in the Department of Gynecology and Obstetrics of the General Hospital during the study period were invited to participate. In total, 146 patients suffering from hypertensive disorders in pregnancy were included in the study. All of them resided in Durango City. In total, 146 cases were enrolled in the study. Of them, 27 had mild preeclampsia, 95 severe preeclampsia, 16 eclampsia, and 8 HELLP syndrome. Mean age of the cases was 23.51 ± 6.41 years (range: 15–39 years).

Control pregnant women

Inclusion criteria for the control group were: 1) pregnant women without hypertensive disorders, diabetes, or nephropathy before or during pregnancy attended in the Department of Gynecology and Obstetrics of the General Hospital in Durango City; 2) to have a normal pregnancy with systolic blood pressure <140 mmHg and diastolic blood pressure <90 mmHg; 3) patients without any underlying disease; and 4) who agreed voluntary participation in the study. Thus, 146 control women were included in this case-control study. Controls were 23.44 ± 6.17 (range: 15–39) years old, and their age was comparable to the one in cases ($P = 0.92$).

General clinical characteristics including age, number of pregnancies, cesarean sections, and deliveries, history of miscarriages and stillbirths, trimester of present pregnancy, history of preeclampsia, systolic and diastolic blood pressures, and mean arterial pressure from all participants were obtained.

Laboratory tests

Serum samples of the participants were kept frozen until analyzed. The presence of anti-CMV antibodies in serum samples was determined by commercially available enzyme immunoassays (EIA). Sera were analyzed for anti-CMV IgG antibodies by the "Cytomegalovirus IgG (CMV IgG)" kit (Diagnostic Automation Inc., Calabasas, CA, USA). In addition, all sera were tested for anti-CMV IgM antibodies by the "Cytomegalovirus IgM (CMV IgM" kit (Diagnostic Automation Inc., Calabasas, CA, USA). All tests were performed following the manufacturer's instructions. The cut-off values for IgG and IgM seropositivity were obtained by firstly multiplying the mean optical densities of IgG and IgM calibrators by the correction factor (0.50) of the calibrator to obtain the corrected mean cut-off value; secondly, the CMV G and M indexes were calculated by dividing the optical density of each sample by the corrected mean cut-off value. A serum sample was considered positive for IgG or IgM antibodies when a CMV G index or a CMV M index was greater than 1.1, respectively. Negative and positive controls were included in each run. Samples positive for IgM by EIA were further tested by a commercially available enzyme linked fluorescent assay (ELFA): "CMV IgM Vidas" (BioMériux, France).

Statistical analysis

We performed the statistical analysis with the aid of the software Epi Info version 7, and SPSS 15.0 (SPSS Inc., Chicago, Illinois). For calculation of the sample size, we used a 95% confidence level, a power of 80%, a 1:1 proportion of cases and controls, a reference seroprevalence of 65.6% [27] as the expected frequency of exposure in controls, and an odds ratio of 2.3. The result of the sample size

calculation was 134 cases and 134 controls. We compared the age values among the groups by the paired Student's *t* test. The Pearson's χ^2 test and the two-tailed Fisher's exact test (when values were small) were used to assess the association between CMV seropositivity and clinical data of the pregnant women. We calculated the odds ratio (OR) and 95% confidence interval (CI), and a *P* value of <0.05 was considered statistically significant.

Ethics statement

We used only archival serum samples and clinical data from a previous study [26]. The original study was approved by the Institutional Ethical Committee of the General Hospital of the Secretary of Health in Durango City, Mexico. A written informed consent was obtained from all participants.

Results

Anti-CMV IgG antibodies were found in 138 (94.5%) of the 146 controls and in 136 (93.2%) of the 146 cases (OR = 0.78; 95% CI: 0.30–2.05; *P* = 0.62). High (>18 IU/ml) levels of anti-CMV IgG antibodies were found in 52 (37.7%) of the 138 seropositive controls and in 47 (34.6%) of the 136 seropositive cases (OR = 0.87; 95% CI: 0.53–1.43; *P* = 0.59). Anti-CMV IgM antibodies were found in 22 (15.1%) of the 146 controls and in 15 (10.3%) of the 146 cases by using EIA. These EIA IgM positive samples were further tested by ELFA. Anti-CMV IgM antibodies were found in 1 (0.7%) of the 146 controls but in none of the 146 cases using ELFA (*P* = 1.0). Seroprevalence of CMV infection was similar among cases regardless the diagnosis: mild preeclampsia (23/27: 85.2%), severe preeclampsia (89/95: 93.7%), eclampsia (16/16: 100%), and HELLP syndrome (8/8: 100%) (*P* = 0.21).

Seropositivity to CMV did not vary with age (*P* = 0.58). In addition, seropositivity to CMV was not associated (*P* > 0.05) with number of pregnancies, history of deliveries, cesarean sections, miscarriages, stillbirths, or a previous preeclampsia (*Table 1*).

Systolic blood pressure (157.98 ± 17.93 mmHg) in CMV positive cases was similar to that (157.00 ± 21.10 mmHg) in CMV negative cases (*P* = 0.86). Diastolic blood pressure (103.42 ± 10.44 mmHg) in CMV positive cases was similar to that (104.00 ± 12.64 mmHg) in CMV negative cases (*P* = 0.86). There was no difference (*P* = 0.96) in the mean arterial blood pressure among seropositive and seronegative cases (121.64 ± 11.77 mmHg and 121.80 ± 14.82 mmHg, respectively).

Discussion

Infection with CMV has been associated with essential hypertension [28, 29]. In a meta-analysis of three studies, researchers found a significant association between CMV and essential hypertension [29]. However, studies on the association between CMV infection and hypertensive disorders in pregnancy have shown conflicting results [20–25]. Therefore, the present study aimed to determine whether CMV infection is associated with hypertensive disorders in a sample of pregnant women in Durango City, Mexico. Our results of tests including qualitative detection of anti-CMV IgG antibodies, quantitative measure of anti-IgG antibody levels, and qualitative detection of

Table 1. Bivariate analysis of clinical characteristics and rates of IgG seropositivity to CMV in cases

Characteristics	Women tested	Seroprevalence of CMV infection		*P* value
	No.	No.	%	
Pregnancies				
1	75	70	93.3	0.25
2	31	28	90.3	
3	18	17	94.4	
4	8	8	100.0	
5	11	11	100.0	
6	1	1	100.0	
7	2	1	50.0	
Trimester of pregnancy				
2	2	2	100.0	1.00
3	144	134	93.1	
Deliveries				
Yes	65	60	92.3	0.75
No	81	76	93.8	

Table 1. *(cont'd)*

Characteristics	Women tested	Seroprevalence of CMV infection		*P* value
	No.	No.	%	
Cesarean section				
Yes	102	97	95.1	0.16
No	44	39	88.6	
Miscarriages				
Yes	25	25	100.0	0.21
No	121	111	91.7	
Stillbirths				
Yes	3	2	66.7	0.19
No	143	134	93.7	
History of preeclampsia				
Yes	21	19	90.5	0.63
No	125	117	93.6	

anti-CMV IgM antibodies indicate that seropositivity to CMV was equally observed in patients suffering from hypertensive disorders of pregnancy and age-matched pregnant women without these disorders attended in the same hospital. Stratification by clinical types of hypertensive disorders did not show a link between CMV and mild preeclampsia, severe preeclampsia, eclampsia, and HELLP syndrome. Thus, our results do not support an association between CMV infection and hypertensive disorders of pregnancy. Our results conflict with those reported in three previous studies. In a Canadian study, researchers found a significant increase of CMV seropositivity and higher anti-CMV IgG antibody levels in women with preeclampsia than normal pregnancy controls [24]. In another study, Xie et al. found that women with early-onset preeclampsia with HELLP syndrome had a significantly higher CMV IgG seropositivity rate than matched normal and non-pregnancy controls [25]. In addition, women with early-onset preeclampsia had higher anti-CMV IgG antibodies than women with late-onset preeclampsia and normal pregnancy [23]. In contrast, our results agree with those reported by other researchers. Soydinc et al. found that anti-CMV IgG and IgM antibodies seropositivities were not significantly different between pregnant women with preeclampsia and healthy pregnant women [20]. In a Chinese study of 52 pregnant women with preeclampsia and 34 women with uncomplicated pregnancy in their third trimester, seroprevalence of recent and long-dated CMV infections was similar in women with preeclampsia and women with normal pregnancy [21]. In a Norwegian study, no evidence of an effect of CMV IgG seropositivity on the likelihood of developing preeclampsia was found [22].

We found a considerable number of serum samples with positive results of anti-CMV IgM antibodies using EIA. False positive results have been reported in anti-CMV IgM antibody tests [30]. Therefore, to increase the specificity of IgM seropositivity, we used two methods to test for anti-CMV IgM antibodies (EIA and ELFA). Results of ELFA yield only one positive sample for IgM antibodies. The sample was from a control women, and thus, no association of this infection marker with hypertensive disorder of pregnancy was found. This result is in line with the lack of association of CMV IgM seropositivity and preeclampsia reported in a Turkish study [20] and a Chinese study [21].

Our study has some limitations. We enrolled a relatively small cohort of pregnant women, and subgroups of hypertensive disorders were also small. Women from only one public hospital were included in the study. Most women attended in the participating hospital have a low socioeconomic status. Therefore, further studies with a larger sample size, including women from several hospitals, with a higher number of participants with several types of hypertensive disorder of pregnancy and with a variety of socioeconomic status, should be conducted.

Conclusions

No serological evidence of an association between CMV infection and hypertensive disorders of pregnancy in patients in a public hospital in Durango City, Mexico was found. Our results conflict with those reported in previous studies. Therefore, further research to elucidate the role of CMV in hypertensive disorders in pregnancy should be conducted.

Funding sources

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Competing interests

The authors declare that no competing interests exist.

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RESEARCH ARTICLE Open Access

Lack of association between *Toxoplasma gondii* exposure and depression in pregnant women: a case-control study

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Abstract

Background: Very little is known about the link of *T. gondii* infection and depression. Through an age-, gender-, and month of pregnancy-matched case-control study, we determined the association of *T. gondii* infection and depression in pregnant women.

Methods: We studied 200 pregnant women with depression and 200 pregnant women without depression attended in a public hospital in Durango City, Mexico. Pregnant women were tested for the presence of anti-*Toxoplasma* IgG antibodies using an enzyme-linked immunoassay (EIA), and IgG seropositive women were further tested for the presence of IgM using an EIA. IgM positivity by EIA was further analyzed by enzyme-linked fluorescence assay (ELFA).

Results: Anti-*T. gondii* IgG antibodies were found in 9 (4.5%) of the 200 cases and in 12 (6.0%) of the 200 controls (OR = 0.73; 95% CI: 0.30–1.79; *P* = 0.50). The frequency of high (>150 IU/ml) anti-*T. gondii* IgG levels was similar in cases and in controls (OR = 1.20; 95% CI: 0.36–4.01; *P* = 0.75). Two women were positive for IgM by EIA but both were negative by ELFA.

Conclusions: We did not find serological evidence of an association between *T. gondii* infection and depression in pregnant women attended in a public hospital in Durango City, Mexico. Since an association of *T. gondii* and depression in pregnancy has been reported in the U.S. previously, further research to elucidate the role of *T. gondii* in prenatal depression should be conducted.

Keywords: *Toxoplasma gondii*, Seroprevalence, Depression, Pregnant women, Case-control study

Background

Toxoplasma gondii (*T. gondii*) is a widely-distributed parasite [1], transmitted to humans by ingestion of raw or undercooked meat containing tissue cysts, and ingestion of food or water contaminated with oocysts shed by cats [2, 3]. Primary infections in pregnant women may result in vertical transmission leading to congenital infections and disease [4, 5]. Although most infections with *T. gondii* are asymptomatic, some infected individuals develop a disease called toxoplasmosis with involvement of eyes, lymph nodes and central nervous system [6, 7]. Immunocompromised individuals infected with *T. gondii* are at risk for a reactivation of the infection leading to a severe disease mainly of the central nervous system [8]. Infection with *T. gondii* has been linked to psychiatric disorders including schizophrenia [9, 10], bipolar disorder, obsessive-compulsive disorder, and addiction [9]. However, the link between *T. gondii* infection and depression is controversial. In a Cuban study of psychiatric patients, those suffering from depressive disorders had the highest frequency of reactivity to the toxoplasmin intradermal test [11]. However, in a population-representative birth-cohort of individuals in Dunedin, New Zealand, *T. gondii* seropositivity

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was not significantly associated with major depression [12]. Similarly, in a meta-analysis of 50 studies into *T. gondii* infection for major psychiatric disorders versus healthy controls, no association between *T. gondii* IgG seroprevalence and major depression was found [9]. The association of *T. gondii* infection and depression in pregnant women has been poorly studied. Groër et al. found that higher anti-*T. gondii* IgG titers in infected women in the USA were related to depression and anxiety during pregnancy [13]. We aimed to determine whether *T. gondii* infection is associated with depression in pregnant women attended in a public hospital in Durango City, Mexico.

Methods

Study design and population studied

We performed an age-, gender-, and month of pregnancy-matched case-control study of 200 pregnant women suffering from depression and 200 pregnant women without depression attended in a public hospital in Durango City, Mexico. This study was performed from March 2015 to February 2016. Inclusion criteria for enrollment of participants were: 1) pregnant women suffering from depression attending prenatal care consultations in the General Hospital of the Secretary of Health in Durango City; 2) aged 13 years and older; and 3) who accepted to participate in the study. Socioeconomic status was not a restrictive criterion for enrollment. Mean age in cases was 23.40 ± 8.36 (range 13–43) years old. Depressed pregnant women had 2–8 months of pregnancy (mean 6.5 ± 1.5 months). As a strategy to screen depression in pregnant women, validated Mexican versions of the Edinburgh postnatal depression scales (EPDS) (Additional file 1) were used in adults [14] and teenagers [15]. Pregnant women who screened positive for depression in the EPDS were further examined by a psychiatrist to confirm depression using the Diagnostic and Statistical Manual of Mental Disorders, Fifth edition criteria [http://www.dsm5.org/Pages/Default.aspx]. Control pregnant women were matched with cases for age. Controls were randomly selected, and they scored negative for depression in the EPDS. Inclusion criteria for enrollment of control pregnant women were: 1) pregnant women without depression attending prenatal consultations in the General Hospital of the Secretary of Health in Durango City; 2) aged 13 years and older; and 3) who accepted to participate in the study. Mean age in control women was 23.01 ± 7.55 (range 13–45) years old. Pregnant women without depression had 2–9 months of pregnancy (mean 6.7 ± 1.5 months). No statistically significant differences in age ($P = 0.62$), and month of pregnancy between cases and controls were found.

Detection of anti-*T. gondii* antibodies

Serum samples from participants were obtained and stored at −20 °C until analyzed. The presence of anti-*T. gondii* IgG antibodies was tested in sera using the commercially available enzyme immunoassay (EIA) kit "*Toxoplasma* IgG" (Diagnostic Automation/Cortez Diagnostics Inc., Woodland Hills, CA, USA). Anti-*T. gondii* IgG antibody levels were expressed as International Units (IU)/ml, and a cut-off for seropositivity of 8 IU/ml was used. Sera positive for anti-*T. gondii* IgG antibodies were further tested for anti-*T. gondii* IgM antibodies by using the commercially available EIA "*Toxoplasma* IgM" kit (Diagnostic Automation/Cortez Diagnostics Inc.). In addition, sera positive for anti- *T. gondii* IgM antibodies by EIA were further analyzed for these anti-*T. gondii* IgM antibodies using the commercially available enzyme-linked fluorescent assay (ELFA) kit "VIDAS Toxo IgM" (BioMérieux, Marcy-l'Etoile, France). All IgG and IgM assays were performed following the instructions of the manufacturers.

Statistical analysis

Analysis was conducted using the software Epi Info 7 and SPSS 15.0 (SPSS Inc. Chicago, Illinois). For calculation of the sample size, we used a 95% confidence level, a power of 80%, a 1:1 proportion of cases and controls, a reference seroprevalence of 6.1% [16] as the expected frequency of exposure in controls, and an odds ratio of 2.8. The result of the sample size calculation was 195 cases and 195 controls. We used the student's *t* test to compare the age among cases and controls. The association of *T. gondii* infection and depression in pregnant women was assessed with the two-tailed Pearson's chi-squared test. Odds ratio (OR) and 95% confidence interval (CI) were calculated, and a P value < 0.05 was considered statistically significant.

Results

Of the 200 cases of depression included in the study, 122 (61.0%) suffered from minor depression, and 78 (39.0%) from major depression. Anti-*T. gondii* IgG antibodies were found in 9 (4.5%) of the 200 cases and in 12 (6.0%) of the 200 controls. The seroprevalence of *T. gondii* infection was similar in cases and in controls (OR = 0.73; 95% CI: 0.30–1.79; $P = 0.50$). Of the 9 anti-*T. gondii* IgG positive cases, 6 (66.7%) had IgG levels higher than 150 IU/ml, one (11.1%) between 100 and 150 IU/ml, and 2 (22.2%) between 8 and 99 IU/ml. In contrast, of the 12 anti-*T. gondii* IgG positive controls, 5 (41.7%) had IgG levels higher than 150 IU/ml, one (8.3%) between 100 and 150 IU/ml, and 6 (50.0%) between 8 and 99 IU/ml. The frequency of high (>150 IU/ml) anti-*T. gondii* IgG levels was similar in cases and in controls (OR = 1.20; 95% CI: 0.36–4.01; $P = 0.75$). Seroprevalence of *T. gondii* infection in patients with minor depression (4/122: 3.3%) was comparable to that (5/78: 6.4%) found in patients with major depression ($P = 0.29$). Stratification by age

groups (13–30 years, and older than 30 years) did not show differences ($P > 0.05$) in seroprevalences among cases and controls (3/149: 2.0% versus 10/159: 6.3%, and 6/51: 11.8% versus 2/41: 4.9%, respectively). Stratification by month of pregnancy groups (2–5 months, and more than 5 months) did not show differences ($P > 0.05$) in seroprevalences (2% versus 8.7%, and 5.4% versus 5.2%, respectively) or high IgG antibody levels (2% versus 4.3%, and 3.4% versus 1.9%, respectively) among cases and controls. None of the 9 anti-*T. gondii* IgG seropositive cases was reactive to anti-*T. gondii* IgM antibodies by EIA. Whereas 2 of the 12 anti-*T. gondii* IgG seropositive controls were reactive to IgM by EIA. These 2 IgM positive sera by EIA were negative to IgM by ELFA. Thus, none of the cases and controls was considered seropositive to IgM.

Discussion

Studies about the association of *T. gondii* infection and depression have shown conflicting results [9, 11, 12]. In addition, the association of *T. gondii* infection and prenatal depression has been poorly studied in particular. Therefore, the present study aimed to determine whether *T. gondii* infection is associated with depression in a sample of pregnant women in Durango City, Mexico. Results of tests for detection of *T. gondii* performed in the present study included qualitative and quantitative IgG and IgM assays. Our results do not point towards an increased rate of depression in pregnant women infected with *T. gondii* compared to matched control patients attended in the same hospital. We are aware of only one study on the link of *T. gondii* infection and depression in pregnancy. In such study, 414 women at 16–25 weeks of gestation in the USA were examined, and researchers found that higher *T. gondii* IgG antibody titers were associated with prenatal depression [13]. Authors hypothesized that immune escape of *T. gondii* may occur due to immune changes in pregnancy, and this could cause depression through activation of indoleamine 2, 3-dehydroxylase resulting in serotonin decrease [13]. It is not clear why the association of infection and depression was found in pregnant women in the USA but not in pregnant women in the current study. Comparison of both studies was based on IgG seropositivity; however, IgM seropositivity was not compared because this marker for acute or recent infection was not determined in the American study. It is possible that differences in the characteristics of pregnant women among the studies may explain the differences in the association. In the U.S. study, researchers examined women at 16–25 weeks of pregnancy whereas we examined women at 2–9 months of pregnancy. Stratification by month of pregnancy groups (2–5 months, and >5 months) did not show an association of infection and prenatal depression in the current study. While our study did not find any association of infection with *T. gondii* and depression in pregnant women several studies have demonstrated a link between *T. gondii* infection and depression. *T. gondii* seropositivity correlated with the Center for Epidemiologic Studies Depression score, Profile for Mood States-depression, and total mood disturbance score in a study of women veterans in the USA [17]. The age of women in the latter study was higher than in our study. Experimental evidence exists that reactivation of chronic *T. gondii* infection in mice by an immunosuppressive regimen caused depression-like behaviors, specifically, reduced sucrose preference, and increased immobility in the forced-swim test [18]. Researchers of the latter study also observed an enhanced tryptophan catabolic shunt and serotonin turnover that may be involved in the development of the depressive-like behaviors [18]. Reactivation of latent infection in humans is often observed in immunocompromised patients leading to life-threatening toxoplasmic encephalitis; it is therefore difficult to study an association with depression.

False positive results have been reported in anti-*T. gondii* IgM antibody tests [19]. Therefore, to increase the specificity of IgM seropositivity, we used two methods to test for anti-*T. gondii* IgM antibodies (EIA and ELFA). No acute cases of *T. gondii* infection were found, and therefore, treatment against *T. gondii* in the pregnant women studied was not needed.

Our study has limitations. First, we investigated the association of infection with *T. gondii* and depression in a relatively small cohort of pregnant women attending a public hospital. Therefore, our results cannot be extrapolated to pregnant women with different social status, i.e., those attended in private hospitals or other public hospitals. The great majority of women attended in the participating hospital had a low socioeconomic status.

Conclusions

We did not observe serological evidence of an association between *T. gondii* infection and depression in pregnant women attended in a public hospital in Durango City, Mexico. Our results conflict with those reported in a previous study in the USA therefore warranting further research to elucidate the role of *T. gondii* in prenatal depression.

Additional file

Additional file 1: Tool used to screen depression. (DOCX 15 kb)

Abbreviations

CI: Confidence interval; EIA: Enzyme-linked immunoassay; ELFA: Enzyme-linked fluorescence assay; EPDS: Edinburg postnatal depression scale; IU: International units; ml: Milliliter; OR: Odds ratio; SPSS: Statistical package for the Social Sciences; USA: United States of America

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Authors' contributions
CAE, LFSA, and JHT designed the study protocol, performed the data analysis and wrote the manuscript. ALMM, and JMCO obtained blood samples, submitted the questionnaires, and performed the data analysis. CSM and ASA performed the clinical assessment of participants. OL performed the data analysis, and wrote the manuscript. CAE, AASC, JMSP, and EIAS performed the laboratory tests. All authors read and approved the final version of the manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
The Ethics Committee of the General Hospital of the Secretary of Health in Durango City, Mexico approved this study, and written informed consents were obtained from all participants and from the next of kin of minor participants.

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Lack of Association Between *Toxoplasma gondii* Infection and Diabetes Mellitus: A Matched Case-Control Study in a Mexican Population

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Abstract

Background: Very little is known about the association between infection with Toxoplasma gondii (*T. gondii*) and diabetes mellitus. We perform an age- and gender-matched case-control study to determine the association of *T. gondii* infection and diabetes mellitus.

Methods: Cases included 156 patients with diabetes mellitus and 156 controls without diabetes mellitus who attended in two public clinics in Durango City, Mexico. Sera of cases and controls were tested for the presence of anti-*Toxoplasma* IgG and IgM antibodies using commercially available enzyme-linked fluorescence assays (ELFA).

Results: Anti-*T. gondii* IgG antibodies were found in 10 (6.4%) of the 156 cases and in five (3.2%) of the 156 controls (odds ratio (OR): 2.06; 95% confidence interval (CI): 0.69 - 6.19; P = 0.18). The frequency of high (> 150 IU/mL) anti-*T. gondii* IgG levels in seropositive cases (1/10: 10.0%) was comparable to the one (1/5: 20%) in seropositive controls (OR: 0.44; 95% CI: 0.02 - 9.03; P = 1.00). None of the 10 cases and five controls with seropositivity to anti-*T. gondii* IgG antibodies were positive for anti-*T. gondii* IgM antibodies. Stratification by gender showed similar frequencies of *T. gondii* infection in female cases (7/107: 6.5%) and female controls (4/107: 3.7%) (OR: 1.80; 95% CI: 0.51 - 6.34; P = 0.53), and in male cases (3/49: 6.1%) and male controls (1/49: 2.0%) (OR: 3.13; 95% CI: 0.31 - 31.19; P = 0.61).

Conclusions: We conclude that there is not serological evidence of an association between *T. gondii* infection and diabetes mellitus in the studied subjects in Durango City, Mexico. Further studies to elucidate the role of *T. gondii* in diabetes should be conducted.

Keywords: *Toxoplasma gondii*; Seroprevalence; Diabetes mellitus; Case-control study

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Introduction

Toxoplasma gondii (*T. gondii*) is a coccidian parasite causing infections all around the world [1]. There are two main routes of *T. gondii* transmission to humans: ingestion of food or water contaminated with oocysts shed by *T. gondii* infected cats, and eating raw or undercooked meat containing tissue cysts [2, 3]. Vertical transmission may also occur when a primary infection is acquired during pregnancy [2, 4]. Most *T. gondii* infections are asymptomatic [5]. Some infected individuals may develop clinical manifestations of toxoplasmosis including lymphadenopathy, chorioretinitis, and meningoencephalitis [2, 5, 6]. A reactivation of a *T. gondii* infection in immunocompromised patients may lead to a life-threatening disease with involvement of the central nervous system [2, 5]. After infection, *T. gondii* spreads to many organs of the host [7]. The presence of *T. gondii* infection in pancreas has been reported in humans and animals. In humans, *T. gondii* infection may cause pancreatitis [8]. In a series of 18 autopsy cases of acquired toxoplasmosis in New York City, three cases had dissemination of *T. gondii* to pancreas [9]. In fatal toxoplasmic pancreatitis in AIDS patients, autopsies demonstrated pancreatic necrosis with free parasitic forms or tachyzoites [10], and cysts [11]. In animals, fatal acute toxoplasmosis involving pancreas has been observed in experimentally infected mice [12], a naturally infected Valley quail [13], a sand fox [14], and 11 sugar gliders [15].

It is unclear whether involvement of pancreas during *T. gondii* infection may lead to diabetes mellitus. In a Korean study, diabetes mellitus was a major coincidental disease in

T. gondii IgG seropositive patients [16]. In contrast, significantly lower levels of antibodies against *T. gondii* were found in Colombian patients with type 1 diabetes mellitus [17]. In a prospective cohort of Latino elderly in the USA, individuals seropositive to *T. gondii* did not show an increased rate of diabetes [18]. In an age- and gender-matched case-control study of 91 diabetic cases and 93 healthy non-diabetic controls in Iran, researchers found a higher seroprevalence of IgG antibodies against *T. gondii* in diabetic patients than in healthy controls [19]. In a meta-analysis of studies on the association between chronic toxoplasmosis and diabetes mellitus, researchers found that chronic toxoplasmosis was a possible risk factor for type 2 diabetes mellitus, and no association between *T. gondii* and type 1 diabetes mellitus [20]. Since results of studies about the association of *T. gondii* infection and diabetes mellitus are controversial, we decided to perform a matched case-control study to determine whether *T. gondii* seropositivity is associated with diabetes mellitus in subjects attending in a public health institution in Durango City, Mexico.

Materials and Methods

Study design and populations studied

We performed an age- and gender-matched case-control study of 156 patients with diabetes mellitus and 156 individuals without diabetes mellitus. Patients were enrolled in a public clinic for diabetes care (Clinica de Diabetes), and controls were enrolled in a public health center (Centro de Salud de Servicios Ampliados 450) in Durango City, Mexico. Both clinics belong to the same health institution (Secretaria de Salud). This study was performed from February 2015 to March 2017. Inclusion criteria for enrollment of cases were: 1) individuals with diabetes mellitus attending in a public diabetes care center (Clinica de Diabetes) in Durango City, Mexico; 2) aged 18 years and older; and 3) who accepted to participate in the study. Gender, occupation and socioeconomic status were not restrictive criteria for enrollment. In total, 107 (68.6%) females and 49 (31.4%) males with diabetes mellitus were enrolled in the study. Mean age in cases was 56.0 ± 11.1 (range 18 - 83) years old. Of the 156 patients, 151 had type II diabetes and five had type I diabetes. Control individuals were matched with cases for age and gender. Control individuals were randomly selected. Inclusion criteria for enrollment of controls were: 1) individuals without diabetes mellitus attending in public health center of the Secretary of Health (Centro de Salud de Servicios Ampliados 450); 2) aged 18 years and older; and 3) who accepted to participate in the study. The control group included 107 (68.6%) females and 49 (31.4%) males. Mean age in control subjects was 55.5 ± 11.8 (range 20 - 85) years old. Cases and controls had similar age (P = 0.70).

Detection of anti-*T. gondii* IgG and IgM antibodies

Serum samples from cases and controls were obtained and kept frozen at -20 °C until analyzed. Serum samples were analyzed for anti-*T. gondii* IgG antibodies using the commercially available enzyme-linked fluorescent assay (ELFA) kit "VIDAS Toxo IgG II" (BioMerieux, Marcy-l'Etoile, France). All serum samples with positive results in the IgG ELFA were further tested for anti-*T. gondii* IgM antibodies using the commercially available ELFA kit "VIDAS Toxo IgM" (BioMerieux, Marcy-l'Etoile, France). Both IgG and IgM ELFA were performed following the manufacturer's instructions.

Statistical analysis

Analysis of data was performed using the software Epi Info 7 and SPSS 15.0 (SPSS Inc., Chicago, IL). For the sample size calculation, we used the following values: a 95% confidence level, a power of 80%, a 1:1 proportion of cases and controls, and a reference seroprevalence of 6.1% [21] as the expected frequency of exposure in controls. Thus, a sample size of 155 cases and 155 controls was obtained. We used the Student's *t*-test to compare age values among cases and controls. The association between *T. gondii* infection and diabetes mellitus was analyzed with the two-tailed Pearson's Chi-squared test or the Fisher exact test (for small values). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated, and statistical significance was set at a P value less than 0.05.

Ethics aspects

The Ethics Committee of the General Hospital of the Secretary of Health in Durango City, Mexico approved this study. Participation in the study was voluntary, and a written informed consent was obtained from all participants.

Results

Anti-*T. gondii* IgG antibodies were found in 10 (6.4%) of the 156 cases and in five (3.2%) of the 156 controls. The seroprevalence of *T. gondii* infection in cases was similar to the one in controls (OR: 2.06; 95% CI: 0.69 - 6.19; P = 0.18). Of the 10 anti-*T. gondii* IgG positive cases, one (10.0%) had IgG levels higher than 150 IU/mL, and nine (90.0%) between 8 and 99 IU/mL. Whereas, of the five anti-*T. gondii* IgG positive controls, one (20.0%) had IgG levels higher than 150 IU/mL, and four (80.0%) between 8 and 99 IU/mL. The frequency of high (> 150 IU/mL) anti-*T. gondii* IgG levels in cases was similar to the one in controls (OR: 0.44; 95% CI: 0.02 - 9.03; P = 1.00). None of the 10 cases and five controls with seropositivity to anti-*T. gondii* IgG antibodies were positive for anti-*T. gondii* IgM antibodies by ELFA. Stratification by gender showed similar frequencies of *T. gondii* infection in female cases (7/107: 6.5%) and female controls (4/107: 3.7%) (OR: 1.80; 95% CI: 0.51 - 6.34; P = 0.53). The frequency of *T. gondii* infection in male cases (3/49: 6.1%) was similar to the one in male controls (1/49: 2.0%) (OR: 3.13; 95% CI: 0.31 - 31.19; P = 0.61). Of the 151 patients with type II diabetes, nine (6.0%) were positive to IgG against *T. gondii*, whereas one (20%) of the five patients

with type I diabetes had IgG antibodies against *T. gondii* (P = 0.28).

Discussion

Whether *T. gondii* infection is associated with diabetes mellitus is still a matter of controversy. A limited number of studies about this association exist, and have reported conflicting results. Therefore, we sought to determine whether *T. gondii* infection is associated with diabetes mellitus in a sample of adult patients attending in a public clinic for diabetes care in Durango City, Mexico. For this purpose, we assessed the frequency of IgG and IgM antibodies against *T. gondii*, and the anti-*T. gondii* IgG antibody levels. Results of the current study indicate that patients with diabetes mellitus have equal frequencies of IgG and IgM antibodies against *T. gondii*, and anti-*T. gondii* IgG antibody levels as age- and gender-matched control subjects without diabetes. Therefore, our findings based on serological methods do not support an association between diabetes mellitus and *T. gondii* infection. Our results agree with the low (9%) seroprevalence of *T. gondii* infection reported in Mexican Americans from San Antonio Texas, USA that suffered from high rates of obesity and type 2 diabetes mellitus [22], and with low levels of antibodies against *T. gondii* found in Colombian patients with type 1 diabetes mellitus [17]. In addition, our results are in line with the lack of association between *T. gondii* infection and incident diabetes found in a prospective cohort of Latino elderly in New York, USA [18]. On the other hand, findings in favor of an association between *T. gondii* seropositivity and diabetes mellitus include a high seroprevalence of *T. gondii* infection found in patients with diabetes mellitus in general hospitals in Daejeon, Korea [16], and a significantly higher seroprevalence of *T. gondii* infection in patients with diabetes than in healthy controls found in an age- and gender-matched case-control study in Iran [19]. In addition, a meta-analysis of seven studies about the association of chronic toxoplasmosis and diabetes mellitus concluded that chronic toxoplasmosis was a possible risk factor for type 2 diabetes mellitus [20].

For a fair comparison of our results obtained under an age- and gender-matched case-control study design, we searched for similar studies in the medical literature. We were able to find only one age- and gender-matched case-control study about the association of *T. gondii* seropositivity and diabetes mellitus. In such study, researchers found a positive association between *T. gondii* infection and diabetes in Iran [19]. It is not clear why there were differences in the associations among the studies. It is likely that differences in the characteristics of the studied populations among the studies might explain the differences in the association. For instance, differences in age and gender among the studied populations might influence the seroprevalence of *T. gondii* infection. We could not compare the age and gender variables of our studied population with those of the Iranian study because no description of these variables was found in the Iranian study. A clear difference among the studies was the laboratory methods used. We used ELFA to determine antibodies against *T. gondii* whereas an enzyme-linked immunosorbent assay was used in the Iranian study [19]. Sensitivity and specificity of the IgG tests used in the studies are comparable. A 100% sensitivity and a 99% specificity of the enzyme-linked immunosorbent assay used were reported in the Iranian study [19]. According to the insert, the ELFA used in our study has a 99.65% sensitivity and a 99.92% specificity. The numbers of cases and controls were different among the studies. We enrolled 156 cases and 156 controls whereas 91 cases and 93 controls were included in the Iranian study [19].

The present study has limitations: we studied only diabetic outpatients from a single public clinic for diabetes care. The great majority of patients attending in this clinic have low socioeconomic status. The severity of diabetes in outpatients may be milder than in inpatients. Therefore, additional studies with diabetic inpatients, of diverse socioeconomic status, in several clinics should be conducted to further determine the association between *T. gondii* infection and diabetes mellitus.

Conclusions

We conclude that there was not serological evidence of an association between *T. gondii* infection and diabetes in adult patients attending in a public diabetes care center in Durango City, Mexico. Further studies to elucidate the role of *T. gondii* in diabetes should be conducted.

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Competing Interests

The authors declare that no competing interests exist.

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RESEARCH ARTICLE Open Access

The relationship between blood lead levels and occupational exposure in a pregnant population

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Abstract

Background: Pregnant women exposed to lead are at risk of suffering reproductive damages, such as miscarriage, preeclampsia, premature delivery and low birth weight. Despite that the workplace offers the greatest potential for lead exposure, there is relatively little information about occupational exposure to lead during pregnancy. This study aims to assess the association between blood lead levels and occupational exposure in pregnant women from Durango, Mexico.

Methods: A cross-sectional study was carried out in a population of 299 pregnant women. Blood lead was measured in 31 women who worked in jobs where lead is used (exposed group) and 268 who did not work in those places (control group). Chi-square test was applied to compare exposed and control groups with regard to blood lead levels. Odds ratio (OR) and 95% confidence intervals (CI) were calculated. Multivariable regression analysis was applied to determine significant predictors of blood lead concentrations in the exposed group.

Results: Exposed women had higher blood lead levels than those in the control group (4.00 ± 4.08 μg/dL vs 2.65 ± 1.75 μg/dL, $p = 0.002$). Furthermore, women in the exposed group had 3.82 times higher probability of having blood lead levels ≥ 5 μg/dL than those in the control group. Wearing of special workwear, changing clothes after work, living near a painting store, printing office, junkyard or rubbish dump, and washing the workwear together with other clothes resulted as significant predictors of elevated blood lead levels in the exposed group.

Conclusions: Pregnant working women may be at risk of lead poisoning because of occupational and environmental exposure. The risk increases if they do not improve the use of protective equipment and their personal hygiene.

Keywords: Blood lead, Occupational exposure, Pregnant women, Risk factors

Background

Lead has been clearly shown to be a neurotoxic agent widely distributed in the environment [1]. Excessive lead exposure may occur in the workplace. Some jobs that expose people to lead include: mining, smelting, foundry work, construction, plumbing, radiator manufacturing, lead-acid battery recycling, manufacturing of rubber products, and the chemical industry. Years ago, lead was also used regularly in paint, ceramics, and pipe solder among other things. Because of its potential health problems, the amount of lead used in these products today has lessened or has been removed. However, lead is still common in many industries, including construction, mining, and manufacturing [2].

Lead can harm many of the body's organ systems. Human exposure to lead can result in a wide range of biological effects [3]. It is well known that childhood and

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pregnancy are the most sensitive population to lead exposure. A pregnant woman with an elevated blood lead concentration may expose her fetus to the toxic effect of lead. Elevated blood lead levels (BLLs) in children cause learning and behavioral deficits [4, 5]. Low-level lead exposure, including prenatal exposure, has been linked to decreased performance on IQ tests for school children [6–9]. Several studies have suggested that any level of exposure is potentially detrimental and no threshold for these effects has been identified [10, 11].

Lead concentrations have declined in the last decades due to the increase in health interventions [12]. In spite of this, lead exposure remains a risk factor for female reproductive health, even at low levels of lead in blood [13]. Once absorbed from the gastrointestinal tract or the respiratory system, lead is transported bound to erythrocytes and accumulates in bone [14]. During pregnancy, calcium demands increase. This leads to increased bone turnover, with a consequential release of lead from bone and increased blood lead levels [15, 16]. Lead can cross the placenta and expose the fetus to the harmful effects of this toxic, thus affecting the embryonic development of multiple organs and causing neurobehavioral impairments in infancy and early childhood [4, 5, 9, 17]. Therefore, pregnancy is considered a critical time for exposure to lead for the mother and the fetus [14, 18].

Over the past several decades there has been a remarkable reduction in environmental sources of lead and a decreasing trend in the prevalence of elevated blood lead levels [2]. However, some reproductive health damages at levels of lead in blood below 10 μg/dL have been reported. Therefore, in recent years, many studies have focused on the health effects at low levels of lead in blood. Low blood lead concentrations in pregnant women have been associated with miscarriage [19, 20], pregnancy hypertension, or preeclampsia [12, 21–24] premature delivery [13], premature rupture of the membranes [25], and low birth weight [26, 27]. On the other hand, it is considered that lead-related toxicity can occur at levels as low as 5 μg/dL [28]. Hence, maternal exposure to lead plays an important role in adverse pregnancy outcomes.

Despite that the workplace offers the greatest potential for lead exposure, there is relatively little information about the occupational exposure to lead during pregnancy. It is necessary to identify sources of lead exposure relevant to this population. Some of the jobs that commonly involve lead exposure are battery manufacture or repair; construction (welding or cutting lead-painted metal); radiator manufacture or repair; wire cable cutting and manufacture, and cable, battery, or scrap metal salvage, plating operations; manufacturing or using leaded paints, dyes or pigments, or lead soldering in the electronics industry, among others [29]. In Mexico, and in other developing countries, it is common to find pregnant women working in places with potential sources of lead exposure. The aim of this study was to assess the association between blood lead levels and occupational exposure in pregnant women from Durango, Mexico.

Methods

Study population

From June 2007 to May 2008 a cross-sectional study was conducted to evaluate the association between BLLs and some risk factors in pregnant women who received health attention in the State of Durango, Mexico [30]. The study population consisted of pregnant women who received medical attention in two sanitary jurisdictions pertaining to the Secretary of Health. The total estimated number of pregnant women seen in these two jurisdictions during a 1 year period was obtained from the Secretariat of Health databases, and the sample required was distributed equally in 12 municipalities. The participants were recruited from Obstetrics and Gynecology Departments of the municipal hospitals. All women who presented for prenatal care on the days that the study team visited, independent of their gestational age, were asked to participate in the study if they met the inclusion criteria. The inclusion criteria were: being pregnant, living in Durango, able to understand Spanish, and receiving health care paid for by the Secretary of Health. Each municipality was visited two or three times during the recruitment period, until the sample size was completed. Of the 337 pregnant women who presented for prenatal care on the days of the visits, 12 women were excluded because they did not live in Durango and 26 declined to participate in the study. A total of 299 women were included in the study (Aditional file 1). The interviewer's interaction with patients was standardized. All patients gave their informed written consent and answered a set of questions in a face-to-face interview. The research protocol was approved by the Ethical Committee of Durango General Hospital.

First, the group was treated as a cohort. After that, a regression with lead levels as outcome allowed to attribute the proportion of risk from occupational and non-occupational exposure. For assessment of the association between blood lead levels and occupational exposure, subjects were classified into two groups: women who worked in places where lead is used (exposed group) and women who did not work in those places (control group). Women who worked in automotive repair shops, mining laboratories, welding workshops, automotive harness factories, hairdressing salons, and road sweepers were included in the exposed group. Unemployed women and those women who had a job where lead-containing materials are not used, were included in the control group.

Blood lead measurement

Blood samples were collected using lead-free tubes containing EDTA. Samples were stored in the original tube at 4° C before being transferred to the Environmental Toxicology Laboratory, Faculty of Medicine, Juarez University of Durango State. The time between receipt and analysis varied from 1 to 3 weeks. During which time, the specimens were stored refrigerated at 4 °C. Lead concentration was determined by graphite furnace atomic absorption spectrometry. Bovine blood obtained from the National Institute of Standards and Technology (NIST) was used as standard reference material.

Statistical analysis

Data were analyzed to describe demographic characteristics, BLLs, and potential sources of lead exposure. The normality of the variables was tested using the Kolmogorov-Smirnov test. BLLs were log-transformed prior to analysis. Multivariable regression analysis was conducted to determine the proportion of risk from each occupational and non-occupational exposure. After that, the study population was divided into two groups according to occupation (occupationally exposed and non-occupationally exposed). Student *t*-test was applied for comparison of quantitative variables. Chi-square test was applied to compare exposed and control groups regarding blood lead levels (BLLs ≥ 5 μg/dL vs BLLs < 5 μg/dL). Odds ratio (OR) and 95% confidence intervals were calculated. To identify non-occupational sources of lead exposure for pregnant women we explored the following: the way in which workwear is washed (together with other clothes or alone), use of lead-glazed pottery, use of hair dyes, living near workplaces where lead is used (mining zones, battery workshops, junkyards, rubbish dumps and painting workshops), pica behavior and living with someone who works with lead, in both exposed and control groups. These activities have been documented to be lead-related. Chi-square test was also used to compare both groups regarding non-occupational sources of lead exposure. Student *t*-test was also used to compare blood lead levels according to some protection habits in the exposed group. Use of respiratory protective equipment, habit of wearing gloves, wearing of special workwear, handwashing before eating, changing clothes after work, and use of any protective equipment were analyzed as dichotomous variables. Finally, backward stepwise multivariable regression analysis was applied to determine significant predictors of blood lead concentrations in the exposed group. A set of variables selected on the basis of previous knowledge or because of associations with lead levels in bivariate analyses (at $p < 0.25$) were entered into the model. The full model was followed by stepwise backward elimination to determine whether each variable remained significant after non-significant covariates were excluded. All statistical analyses were performed using SPSS for Windows statistical package version 15.0. A p-value < 0.05 was considered statistically significant.

Results

The mean blood lead concentration in the study population was 2.79 μg/dL (SD 2.14), geometric mean 2.38 μg/dL, 95% CI (2.25 – 2.54). Among the 299 pregnant women enrolled in the study, 31 (10.4%) worked in places where lead is used, and 268 (89.6%) did not work where lead-containing materials are used (Table 1). Results of multiple linear regression on association between blood lead levels and risk factors are shown in Table 2. Living in a mine zone was associated with increased blood lead ($p = 0.044$). However, working in places where lead is used was the main factor associated with blood lead concentration. On the basis of this result, the study population was divided into two groups: exposed and non-exposed.

Table 3 summarizes the main characteristics of both groups. There were no significant differences between the groups regarding age, gestational age, number of pregnancies, body mass index (BMI), hemoglobin and monthly income per person. However, the blood lead concentration of the exposed group was significantly higher than that of the control group ($p = 0.002$).

Frequency of BLLs ≥ 5 μg/dL is depicted in Table 4. The proportion of women with BLLs ≥ 5 μg/dL in the exposed group was significantly higher compared to the control group (22.6% vs 7.1%; $p < 0.01$). In addition, women in the exposed group had 3.8 times more

Table 1 General information and blood lead levels of study population ($N = 299$)

Variables	Percent	Mean (SD)
Age (years)		24.32 (6.71)
Gestational age (weeks)		24.07 (8.68)
Pregnancies		2.0 (1.0)
Body mass index (kg/m^2)		27.23 (5.63)
Hemoglobin (g/dL)		12.55 (1.34)
Monthly income per person, USD		140.95 (144.73)
Working in places where lead is used		
Yes	10.4	
No	89.6	
Blood lead levels (μg/dL)		2.79 (2.14)
Geometric mean (95% CI)		2.38 (2.25 – 2.54)

Table 2 Results from the multiple linear regression analysis on the association between blood lead and risk factors

Risk factor	Coefficient β	95% CI	*p*
Washing the workwear together with other clothes	0.106	- 0.018 – 0.229	0.093
Use of lead glazed pottery	0.033	- 0.102 – 0.168	0.634
Dyeing hair	- 0.016	- 0.147 – 0.115	0.813
Living near workplaces where lead is used	- 0.021	- 0.197 – 0.156	0.818
Living near mining zone	0.237	0.006 – 0.468	0.044
Living near battery workshop	- 0.016	- 0.209 – 0.177	0.869
Living near junkyard	- 0.079	- 0.284 – 0.127	0.452
Living near rubbish dump	0.141	- 0.060 – 0.342	0.169
Living near straightening and painting workshop	0.023	- 0.172 – 0.218	0.819
Pica behavior	0.115	- 0.032 – 0.261	0.124
Living with someone who works with lead	0.056	- 0.071 – 0.183	0.387
Living near painting store	0.081	- 0.167 – 0.329	0.521
Living near printing office	- 0.120	- 0.441 – 0.201	0.461
Working in places where lead is used	0.306	0.103 – 0.509	0.003

R2 = 0.082

probability to have BLLs above 5 μg/dL than those in the control group.

Non-occupational sources of lead exposure for exposed and control groups are summarized in Table 5. The proportion of women who had the habit of dyeing their hair was significantly higher in exposed women when compared to the control group ($p = 0.010$) and the same was observed in the exposed group regarding living near workplaces where lead is used when compared with control women ($p = 0.043$). However, there were no significant differences in other variables between the compared groups.

To evaluate the influence of some work conditions on blood lead levels in the exposed group, some protection habits were explored (Table 6). Blood lead levels were significantly higher in women who did not wear special workwear ($p = 0.028$) and in those who did not have the habit of changing clothes after work ($p = 0.025$).

Table 7 displays potential sources of blood lead in the exposed group. After multivariable analysis, seven variables were retained in the final model: wearing of special workwear, changing clothes after work, living near a painting store, living near a printing office, living near a junkyard, living near a rubbish dump and washing the workwear together with other clothes. These variables accounted for 86.5% of the total variance. The model was adjusted by age, educational level and gestational age.

Discussion

In this cross-sectional study, we examined the association of blood lead levels with occupational exposure in pregnant women. The blood lead levels in our

Table 3 General information and blood lead levels of the exposed subjects and control group[a]

Variable	Exposed group ($n = 31$)	Control group ($n = 268$)	*p* value[*]
Age (years)	26.03 (6.17)	24.13 (6.76)	0.135
Gestational age (weeks)	22.71 (8.06)	24.22 (8.75)	0.358
Number of pregnancies	2.55 (1.38)	2.23 (1.47)	0.253
Body mass index (kg/m^2)	28.81 (4.79)	27.04 (5.70)	0.098
Hemoglobin (g/dL)	12.97 (1.11)	12.50 (1.36)	0.065
Monthly income per person, USD	165.62 (130.59)	138.00 (146.28)	0.316
Blood lead levels (μg/dL)	4.00 (4.08)	2.65 (1.75)	0.002[**]

[a]Values shown as mean (standard deviation)
[*] *p* value was calculated from Student *t*-test
[**] *p* value from Log BLL

Table 4 Frequencies of BLL ≥ 5 μg/dL in the study population

Subjects	BLLs ≥ 5 μg/dL n (%)	BLLs <5 μg/dL n (%)
Exposed group ($n = 31$)	7 (22.6)	24 (77.4)
Control group ($n = 268$)	19 (7.1)	249 (92.9)
Total ($n = 299$)	26 (8.7)	273 (91.3)

$X^2 = 6.56$; $p = 0.010$; OR = 3.822; 95%; IC (1.460 – 10.008)

study population (2.79 ± 2.14 μg/dL) did not exceed the accepted threshold of 10 μg/dL. They are even below the 5 μg/dL recommended by the CDC [31]. Furthermore, the mean blood lead level in our test subjects is lower compared to values reported in some populations of pregnant women. A study by Taylor et al. [14] reported mean BLL of 3.67 ± 1.47 μg/dL in a cohort of pregnant women in The United Kingdom. In China, the lead concentrations during the three pregnancy trimesters and postpartum were 5.95 ± 2.27 μg/dL, 5.51 ± 1.93 μg/dL, 5.57 ± 1.85 μg/dL, and 6.88 ± 1.90 μg/dl; respectively [32]. In addition, Gerhardsson and Lundh [33] reported median blood lead of 11.0 μg/L (range 4.2–79 μg/L) in pregnant females residing in Sweden; and Alvarez et al. [34] found a blood lead average of 11.63 ± 4.64 μg/dL in pregnant women living in the island of Tenerife, Spain. However, some researchers have reported lower blood lead concentrations in pregnant women. Mean blood lead levels of 2.551 ± 2.592 μg/dL were found in pregnant women from Saudi Arabia [35]. In a socioeconomically disadvantaged population of New York, a geometric mean of 1.58 μg/dL was reported by Schell et al. [15]. Moreover, Bakhireva et al. [36] found mean blood lead of 1.06 ± 1.55 μg/dL in a cross-sectional study designed to ascertain risk factors of lead exposure among pregnant women in New Mexico, United States.

In Mexico, the Secretary of Health is the health care institution which attends the smallest workforces. Nevertheless, we found 31 women working in places where lead is used and who represent 10.4% of the recruited subjects. In spite of this, lead in the workplace results a significant determinant of blood lead levels. Therefore, similar results may be expected in other pregnant populations with low income and low level of employment.

Our exposed group was made up of women who worked in automotive repair shops, mining laboratories, welding workshops, automotive harness factories, hairdressing salons, and as road sweepers, regardless of intensity and exposure time. At any rate, we found significantly higher blood lead concentrations in exposed women than in the control group (4.24 ± 4.60 μg/dL vs. 2.66 ± 1.73 μg/dL). Our findings are consistent with a study by Popovic et al. [37], who found mean blood lead of 2.73 ± 2.39 μg/dL in women formerly working in a smelter, and 1.25 ± 2.10 μg/dL in women with no known occupational exposure to lead.

In the present study, no difference was observed in hemoglobin level between exposed women and the control group. This is expected considering the low BLLs obtained for this population. According to previous studies, lead anemia appears at BLLs higher than 40 μg/dL [3, 38]. On the other hand, the US Environmental Protection Agency (EPA) suggests a threshold BLL of 20 – 40 μg/dL for risk of anemia [39]. However, blood lead concentrations in our compared groups are much lower.

Table 5 Comparison of non-occupational sources of lead exposure between exposed and control groups[a]

Potential source of lead exposure	Exposed group ($n = 31$)	Control group ($n = 268$)	p value*
Washing the workwear together with other clothes	12 (38.7)	123 (45.9)	0.447
Use of lead glazed pottery	10 (32.3)	81 (30.2)	0.816
Dyeing hair	27 (87.1)	172 (64.2)	0.010
Living near workplaces where lead is used	22 (71.0)	139 (51.9)	0.043
Living near mining zone	4 (12.9)	25 (9.3)	0.752
Living near battery workshop	7 (22.6)	43 (16.0)	0.356
Living near junkyard	4 (12.9)	30 (11.2)	0.777
Living near rubbish dump	3 (9.7)	39 (14.6)	0.641
Living near straightening and painting workshop	7 (22.6)	45 (16.8)	0.421
Pica behavior	10 (32.3)	62 (23.1)	0.261
Living with someone who works with lead	16 (51.6)	101 (37.7)	0.133

[a]Values shown as frequency (percentage)
*p value from Chi-square test

Table 6 Comparison of blood lead levels regarding protection habits in exposed women

Protection habits	Blood lead levels, μg/dL [a]		*p* value*
	No	Yes	
Use of respiratory protective equipment	27 (4.03 ± 4.23)	4 (3.75 ± 3.43)	0.901
Wearing gloves habit	19 (4.32 ± 4.96)	12 (3.48 ± 2.18)	0.521
Wearing of special workwear	20 (4.92 ± 4.85)	11 (2.31 ± 0.68)	0.028
Hand washing before eating	11 (3.55 ± 1.48)	20 (4.24 ± 5.00)	0.571
Changing clothes after work	24 (4.51 ± 4.52)	7 (2.24 ± 0.60)	0.025
Use of any protective equipment	9 (5.64 ± 7.03)	22 (3.32 ± 1.83)	0.356

[a] Values shown as frequency (mean ± standard deviation)
* *p* value from Student *t*-test

Recent findings concerning lead-related adverse reproductive outcomes suggested that pregnant women should avoid lead exposure that would result in blood lead concentrations higher than 5 μg/dL [3]. Among the 299 women included in our study, 26 (8.7%) had BLLs ≥ 5 μg/dL. In a cohort of 4, 285 pregnant women, Taylor et al. [14] reported 14.4% of women with BLLs of 5 μg/dL or higher; cigarette smoking, alcohol, and coffee drinking were found to be predictors of BLLs. However, in our study the frequencies of smoking, alcohol and coffee drinking among the women were very low; therefore, these variables were not included in the analysis. Regarding occupation, the 2005 – 2007 Adult Lead Epidemiology and Surveillance (ALES) by the United States of America Centers for Disease Control and Prevention reported that 32% of women of childbearing age with BLL ≥ 5 μg/dL were occupationally exposed to lead [38]. Zhu et al. [40] evaluated reasons for testing and potential sources of exposure among women, and reported that 29.2% of women with blood lead of 5–14.9 μg/dL had a job with potential lead exposure.

Our results indicated that exposed women were more than 3.8 times likely to have BLLs ≥ 5 μg/dL than non-exposed women. This finding suggests that occupation represents an important factor for elevated blood lead concentrations in our studied population. According to a study by Kosnett et al. [3], it is recommendable for pregnant women to avoid lead exposure that would result in blood lead levels above 5 μg/dL, due to the raised concerns regarding the toxicity of this blood lead concentration. Several studies have associated blood lead levels above 5 μg/dL with miscarriage [19, 20], pregnancy hypertension [12, 21–24, 41], premature delivery [13], premature rupture of the membranes [25], and low birth weight [26, 27]. According to CDC recommendations [28], pregnant women with a current or past BLL ≥5 μg/dL should be assessed for the adequacy of their diet and provided with prenatal vitamins, calcium and iron supplements.

We found a higher proportion of women living near workplaces where lead is used among exposed women compared with the control group. There was also a significant association between the BLLs and the habit of dyeing the hair. Some hair dyes may contain lead and other harmful substances. Our results agree with Marzulli [42] who reported a significant correlation between blood lead and hair lead in people who used lead contained hair dyes. Use of these products by a pregnant woman may harm the health of her unborn child. None of the cited investigations, carried out in an occupational cohort, analyzed non-occupational exposure. However, our findings suggest that the contribution of non-occupational activities must be explored for determining total lead exposure and subsequent health effects.

Table 7 Regression analysis for predictors of BLLs in exposed group (*N* = 31)

Variable	Coefficient β	95% CI	*P**
Wearing of special workwear	- 0.608	- 1.115 – -0.102	0.021
Changing clothes after work	- 0.637	- 1.261 – - 0.013	0.046
Living near painting store	3.937	1.174 – 6.699	0.008
Living near printing office	7.418	.963 – 10.873	0.001
Living near junkyard	3.661	0.691 – 6.632	0.019
Living near rubbish dump	3.469	0.036 – 6.901	0.048
Washing the workwear together with other clothes	2.372	0.267 – 4.477	0.029

$R^2 = 0.865$
* Adjusted by age, educational level and gestational age

Occupational lead exposure can occur because of the use of lead material and products. For that reason, employers should provide their employees with adequate working conditions and protection information regarding hazards at their worksites. Exposed workers should use protective equipment and practice personal hygiene, such as showering and changing into clean clothes at the end of the shift [43]. In this study, working women who did not change their clothes after work showed significantly higher blood lead concentration in comparison with those women who had this habit. There was also statistical association of BLLs related to the use of special workwear. It is well known that appropriate workwear can greatly reduce exposure to hazardous substances [44]. In addition, clothing contaminated with lead can be an important route of exposure for pregnant women.

Despite the scientific data and practical considerations regarding the prevention of lead exposure during pregnancy, routine blood lead testing for pregnant women is not established in many countries. Nevertheless, it is the main way to make sure that women have not been affected by lead. Furthermore, some researchers have demonstrated that lead exposure during pregnancy affects children's physical neonatal development, and available evidence suggests there are no BLLs without risk of health effects [41].

Relatively little is known about the current prevalence, risk factors, and sources of lead poisoning among pregnant women [45]. Our study identified some risk factors associated with blood lead in occupationally exposed women. Despite the growing evidence that relatively low levels of environmental lead exposure may be associated with adverse pregnancy outcomes, there is no specific regulation in existence regarding occupational lead exposure during pregnancy in Mexico. Therefore, it is necessary to improve engineering controls and personal hygiene to reduce the risk of lead exposure during pregnancy. Much work needs to be done to reduce environmental lead exposure. Furthermore, exposed women should undergo blood lead testing to prevent lead poisoning.

We have recognized that our study has several limitations. First, the cross-sectional design did not allow an evaluation of the length and the extent of the exposure. Consequently, all the exposed women were included in a single group, regardless of the time spent in the working place. Longitudinal studies are needed to evaluate the changes in blood lead levels during the exposure time. Second, in our study calcium supplementation, dietary iron intake and indicators of iron status were not measured. It has been documented that low calcium intake may contribute to lead mobilization from the maternal skeleton during pregnancy [46] and that calcium supplementation reduces bone resorption [47] and minimizes release of lead from bone stores with subsequent fetal lead exposure [48, 49]. On the other hand, an inverse relationship between body stores of iron and lead retention has also been observed [50, 51]. Nevertheless, to our knowledge, it is the first study on this topic conducted in occupationally exposed pregnant women in Mexico. Therefore, the results of the present research can be used for comparison with future investigations regarding occupational exposure to lead during pregnancy.

Conclusions

Our results constitute evidence that pregnant women who work in some places where lead products are used may be at risk for presenting higher blood lead levels if they do not use protective equipment and do not practice adequate personal hygiene. The risk increases if women live near some places that are considered sources of lead exposure such as a painting store, a printing office, a junkyard, or a rubbish dump. Additional studies using larger sample sizes and multiple prospective measurements are needed to verify our findings.

Additional file

Additional file 1: Database: Blood lead levels in pregnant women from Durango, Mexico. (XLS 733 kb)

Abbreviations

ALES: Adult lead epidemiology surveillance; BLLs: Blood lead levels; BMI: Body mass index; CDC: Centers for disease control and prevention; CI: Confidence interval; EDTA: Ethylenediaminetetraacetic acid; NIST: National Institute for Standard Technology; OR: Odds ratio; SD: Standard deviation

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Availability of data and material

All data analyzed during this study are included in this published article, in the Additional file 1: DatabasePb.xls.

Authors' contributions

OLLLL designed the study, participated in the elaboration of the questionnaire, prepared the background, results and discussion sections, as well as part of the methods sections, JMSP participated in the elaboration of the questionnaire, he was the field supervisor and contributed to the discussion of results, SEM collaborated in the statistical analysis and interpretation of results, EER carried out part of the literature review, participated in the process of data collection and contributed to the discussion and interpretation of results, FXCJ contributed to the discussion and interpretation of results and prepared part of the results and discussion sections, ASC participated in the elaboration of the questionnaire, in the

process of data collection and critically reviewed the manuscript, AMLQ participated in the design of the questionnaire, carried out part of the literature review and collaborated to the interpretations of results, FVA collaborated in the statistical analysis and interpretation of results, EMMH contributed to the discussion of the findings, she also contributed in drafting and writing of the manuscript, GGV supervised the procedures for blood lead measurements and contributed to the discussion of the findings. JDS contributed with blood lead measurements, interpreting the results, and providing critical comments. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
This study was approved by the Ethical Committee of Durango General Hospital. All participants gave their informed written consent before being enrolled.

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Association between Blood Lead Levels and Delta-Aminolevulinic Acid Dehydratase in Pregnant Women

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Abstract: Blood lead levels (BLLs) and delta-aminolevulinic acid dehydratase (ALAD) activity are considered biomarkers of lead exposure and lead toxicity, respectively. The present study was designed to investigate the association between BLLs and ALAD activity in pregnant women from Durango, Mexico. A total of 633 pregnant women aged 13–43 years participated in this study. Blood lead was measured by a graphite furnace atomic absorption spectrometer. ALAD activity was measured spectrophotometrically. Mean blood lead was 2.09 ± 2.34 μg/dL; and 26 women (4.1%) crossed the Centers for Disease Control (CDC) recommended level of 5 μg/dL. ALAD activity was significantly lower in women with levels of lead ≥5 μg/dL compared to those with BLLs < 5 μg/dL ($p = 0.002$). To reduce the influence of extreme values on the statistical analysis, BLLs were analyzed by quartiles. A significant negative correlation between blood lead and ALAD activity was observed in the fourth quartile of BLLs ($r = -0.113$; $p < 0.01$). Among women with blood lead concentrations ≥2.2 μg/dL ALAD activity was negatively correlated with BLLs ($r = -0.413$; $p < 0.01$). Multiple linear regression demonstrated that inhibition of ALAD in pregnant women may occur at levels of lead in blood above 2.2 μg/dL.

Keywords: blood lead levels; delta-aminolevulinic acid dehydratase (ALAD) activity; pregnant women; lead exposure; lead toxicity

1. Introduction

Lead is known to represent a significant environmental hazard to pregnant women and their offspring. Exposure to high environmental levels of lead during pregnancy has been associated with some adverse outcomes [1]. However, recent findings indicate that lead may be toxic at levels previously considered to have no adverse effects. Research suggests that lead exposure at both low

and high concentrations adversely affects hematopoietic, vascular, nervous, renal and reproductive systems [2]. During pregnancy, adverse reproductive outcomes may occur at levels of lead in blood below 10 μg/dL. Infertility [3], spontaneous abortion [4], preeclampsia [5–7] and preterm delivery [8] have all been associated with lead exposure at levels previously considered safe.

Blood lead concentrations above 2.5 μg/dL have been associated with an increased risk of infertility [3]. A significant association between blood lead concentrations and hypertension during pregnancy has been documented [5,7]. Significantly higher blood lead levels have been reported in women with pregnancy-induced hypertension compared to normotensive patients, and significant correlations between blood lead levels and systolic and diastolic blood pressures have been found [7]. Moreover, higher levels of lead in umbilical cord blood have been found in preeclampsia cases compared to women without this condition [5].

Elevated lead levels have been also associated with abortion and duration of pregnancy [4,8]. In a prospective study in Mexico city a statistically significant relationship between low-to-moderate maternal lead levels and the risk of spontaneous abortion was demonstrated [4]. Furthermore, researchers have found significantly higher blood lead levels during the first trimester of pregnancy in mothers who delivered preterm babies when compared with those whohadfull-term pregnancies [8].

Several biological techniques and biomarkers are useful for risk assessment of lead in the field of environmental health. Blood lead is the most widely used biomarker of lead exposure. This indicator represents a measure of soft tissue lead, body burden and absorbed doses of lead, whereas the critical effects of lead in bone marrow can be used as biomarker of effect. The effects of lead in bone marrow arise mainly from lead interaction with some enzymatic processes involved in heme synthesis [9].

The main biomarkers of effect are the inhibition of delta-aminolevulinic acid dehydratase (ALAD) and the variation in some metabolite concentrations, such as zinc protoporphyrin (ZP) in blood, delta-aminolevulinic acid in urine (ALA-U), delta-aminolevulinic acid in blood (ALA-B), delta-aminolevulinic acid in plasma (ALA-P) and coproporphyrin in urine (CP). However, not all mentioned indicators equally reflect dose and internal dose/effect relationship [2].

Lead toxicity may be explained by its interference with different enzymes. Lead inactivates these enzymes by binding to the SH-groups of proteins or by displacing some essential metal ions. Lead is known to inhibit three enzymes involved in the heme pathway: delta-aminolevulinic acid dehydratase, ferrochelatase, and coproporphyrinogen oxidase, but the major effectsareon ALAD activity. The δ-aminolevulinic acid dehydratase is the second enzyme of the heme pathway. This enzyme catalyzes the condensation of two molecules of δ-aminolevulinic acid (ALA) to form the monopyrrole porphobilinogen (PBG) [10]. In subsequent steeps, PBG is assembled into tetrapyrrole molecules, which constitute the prosthetic groups of hemoglobin [11]. Lead inhibition of ALAD activity results in accumulation of δ-aminolevulinic acid. ALA has been associated with oxidative damage by causing formation of reactive oxygen species (ROS), such as superoxide, hydroxyl radical, and hydrogen peroxide [12–14].

Negative correlations between blood lead concentration and ALAD activity have been reported, even at low levels of lead in blood [9,15,16]. On the other hand, positive correlations have been found between ALAD activity and malondialdehyde (MDA) levels [16]. Thus, ALAD activity is thought to be a sensitive indicator of early effect of lead as well as a biomarker of oxidative stress in the lead-exposed hematological system [17]. Blood lead has been considered a reliable indicator for the evaluation of lead exposure, whereas inhibition of ALAD activity has been considered one of the primary detectable parameters of lead poisoning [2].

Activity of ALAD is easily assayable in samples of peripheral blood. This enzyme has a high sensitivity to divalent lead ions, so it can be used as an indirect biomarker to estimate exposure to lead in humans [18]. ALAD activity test is considered appropriate for screening purposes, due to the progressive inactivation of this enzyme by lead over a range corresponding to subclinical intoxication [19]. In addition, ALAD activity is more sensitive than ALA in urine to evaluate the amount of circulating lead [9,20].

Previous epidemiological studies on the association between blood lead levels (BLLs) and ALAD activity showed divergent views. Studies reporting high levels of lead in blood revealed significant negative correlations between blood lead concentrations and ALAD activity [12,21,22]. However, some authors have demonstrated that ALAD inhibition occurs at levels of lead in blood around 5 μg/dL [15,16,23]. Most studies regarding the association between BLLs and ALAD activity have been conducted in occupationally exposed people and in children. Nevertheless, no significant variation of enzymatic ALAD activity has been reported in children at mean blood lead of 2.58 ± 0.30 μg/dL [13].

In a previous study, conducted by our research group, blood lead levels and some risk factors for lead exposure in pregnant women were determined, but ALAD activity was not evaluated [24]. The present cross-sectional study was designed to investigate the association between BLLs and ALAD activity in pregnant women from Durango, Mexico.

2. Materials and Methods

2.1. Subjects

This cross-sectional study was carried out between January 2014 and June 2016. The study subjects consisted of 633 clinically healthy pregnant women who received prenatal health care by the Secretariat of Health, State of Durango, Mexico. All pregnant women presented for prenatal care in health centers were asked to participate in the study. Those who accepted gave their written informed consent before being enrolled. Patients with renal failure, infectious disease or multifetal pregnancy were excluded. Participants were informed of the aims of the investigation and received information on ways to minimize their lead exposure. Each subject answered a questionnaire that contained sociodemographic data and information on reproductive history and sources of lead exposure. The study was conducted in accordance with the Declaration of Helsinki, and the research protocol was approved by the Ethical Committee of Durango General Hospital (approval number: 366/013).

2.2. Sample Collection

For determination of ALAD activity, a venous blood sample was drawn for each patient and collected in vacutainer tubes using sodium heparin as an anticoagulant. A second sample was collected in lead-free vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA), and separated in two portions; one for hematological analysis, and the remaining aliquot for lead level determination. Blood samples were collected before fasting. After collection, blood samples were transported in ice boxes to the Clinical Analysis Laboratory, Scientific Research Institute, Juarez University of the State of Durango. Samples were stored and transported in a lead-free environment to avoid any contamination, handled by trained personnel and kept in reserve at 4 °C.

2.3. Measurement of ALAD Activity

Enzyme activitywas assayed spectrophotometrically by the standardized European method [25]. The enzyme was incubated with excess δ-aminolevulinic acid at 37 °C. The porphobilinogen which was formed in 1 h was mixed with modified Ehrlich reagent. The color developed was measured spectrophotometrically at 555 nm against a blank. Results were expressed as δ-aminolevulinic acid, μmol/min per liter erythrocytes (U/L). The activity was determined no later than 10 h after the sample collection.

2.4. Hematological Analysis

Hematological parameters were determined using an automated hematology analyzer (Abbott CELL-DIN 1400), at the Clinical Analysis Laboratory, Scientific Research Institute, Juarez Universityof the State of Durango. Red blood cells count (RBC), hemoglobin (Hb), hematocrit, meancorpuscular volume (MCV), mean corpuscular hemoglobin, and meancorpuscular hemoglobin concentration were determined. The hematocrit value was used for the calculation of the enzyme

activity. Only hemoglobin value was presented in the results because of the possible relationship between hemoglobin and blood lead levels.

2.5. Determination of Lead in Blood

Blood samples were transferred to the Laboratory of Environmental Toxicology, Faculty of Medicine, Juarez University of the State of Durango, Gomez Palacio Campus. This laboratory participates in the Wisconsin State Laboratory Program of Hygiene proficiency testing (WSLPHT). Blood lead was measured using a graphite furnace atomic absorption spectrometer Perkin-Elmer AAnalyst 800 with Zeeman-effect background correction. Duplicates of blood samples were analyzed according to Miller et al. [26]. Lead in bovine blood from the National Institute of Standard and Technology (NIST) was used as standard reference material. Each sample duplicate was analyzed twice and those with variation coefficient above 5% were reanalyzed.

2.6. Statistical Analysis

The sociodemographic and reproductive characteristics were shown as mean ± standard deviation. The study population was divided into two groups: those with BLLs < 5 μg/dL and those with BLLs ≥ 5 μg/dL, and Student's *t*-test was used to estimate differences between groups. To reduce the influence of extreme values on the statistical analysis, blood lead levels were analyzed by quartiles. One-way ANOVA was applied to compare the means between quartiles and the post-hoc comparisons were done using Tukey's test. Pearson correlation analysis was carried out to evaluate the relationship of blood lead concentration with hemoglobin and ALAD activity in all groups. Multiple linear regression was performed to evaluate the association of ALAD activity with BLLs. Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) software for Windows, version 15.0. A value of $p < 0.05$ was considered statistically significant.

3. Results

Table 1 summarizes the main characteristics, blood lead levels, and ALAD activity of women enrolled in this study. The mean age, education, gestational age, body mass index and hemoglobin of the studied population were 22.85 years, 10.04 years, 13.44 weeks, 25.61 kg/m^2 and 13.00 g/dL, respectively. The mean income per capita accounted 99.55 United States Dollars (USD) per month (1 USD = 17.0 Mexican pesos). The mean level of blood lead was 2.09 ± 2.34 μg/dL; and the mean ALAD activity was 57.59 ± 21.12 U/L.

Table 1. Main characteristics of the studied subjects (n = 633). ALAD: delta-aminolevulinic acid dehydratase.

Variables	Mean ± SD *	Range
Age (years)	22.85 ± 6.35	13–43
Education (years)	10.04 ± 2.67	0.0–21.0
Gestational age (weeks)	13.44 ± 4.86	3.0–28.0
Body mass index (kg/m^2)	25.61 ± 5.25	16.0–54.4
Income per capita (USD ** per month)	99.55 ± 89.68	4.41–970.59
Hemoglobin, g/dL	13.00 ± 1.27	8.8–23.1
Blood lead levels, μg/dL	2.09 ± 2.34	0.48–26.85
ALAD activity, U/L	57.59 ± 21.12	3.28–138.81

Note: * SD = standard deviation; ** USD = United States Dollars.

Table 2 shows some characteristics for women with lead levels <5 μg/dL, and for women with lead levels ≥5 μg/dL. No significant differences between the groups were observed in age, education, gestational age, body mass index, monthly income per person and hemoglobin. However, ALAD activity was significantly lower in women with lead levels ≥5 μg/dL ($p = 0.002$).

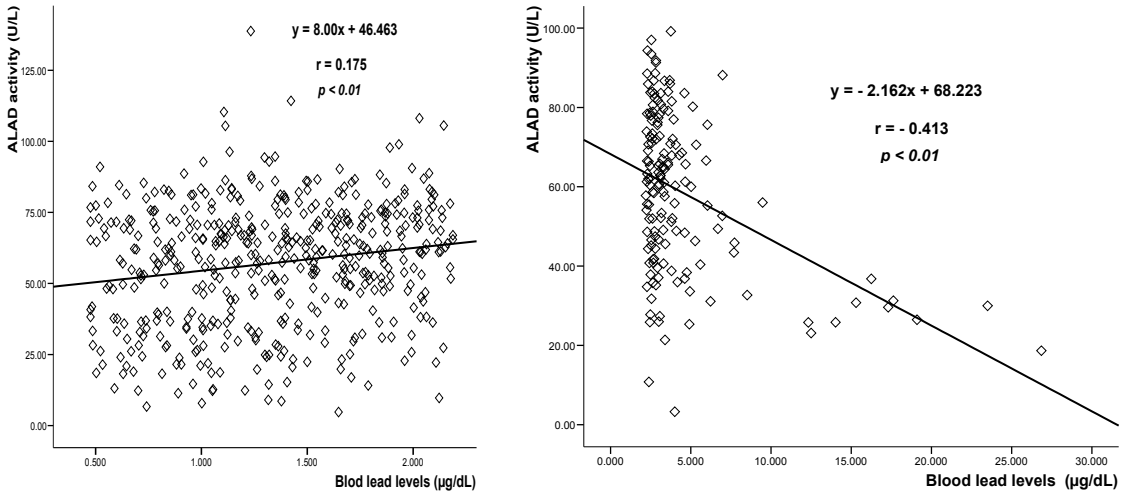
Table 3 shows sociodemographic variables, hemoglobin and ALAD activity by quartiles of blood lead. A significant variation of ALAD activity was observed ($p < 0.001$). According to the Tukey test, women in the first quartile had the lowest ALAD activity. On the other hand, enzyme activity decreased between the third and the fourth quartiles. On the basis of these results, Pearson correlation was performed to determine the relation of blood lead concentration with hemoglobin and ALAD activity by quartiles of BLLs (Table 4). The correlation of BLLs with hemoglobin was not statistically significant. However, significant negative correlation between BLLs and ALAD activity was observed in the fourth quartile (r = −0.413; $p < 0.01$).

Table 2. Main characteristics of women with blood lead levels <5 μg/dL and ≥5 μg/dL. BLL: blood lead levels.

Variables	BLLs < 5 μg/dL (n = 607)	BLLs ≥ 5 μg/dL (n = 26)	*p* *
	Mean ± SD	Mean ± SD	
Age (years)	22.87 ± 6.36	22.42 ± 6.13	0.728
Education (years)	10.06 ± 2.68	9.58 ± 2.52	0.372
Gestational age (weeks)	13.46 ± 4.85	12.95 ± 5.06	0.612
Body mass index (kg/m^2)	25.53 ± 5.20	27.36 ± 5.92	0.082
Income per capita (USD per month)	99.76 ± 78.70	94.32 ± 69.27	0.776
Hemoglobin, g/dL	13.00 ± 1.28	13.00 ± 1.04	0.974
ALAD activity, U/L	58.13 ± 21.05	45.10 ± 19.22	0.002

Note: * *p*-value was calculated from Student's *t*-test.

Taking into account the lower limit of blood lead for the third quartile, linear regression analysis was performed to determine the strength of the relationship between BLLs and ALAD activity in women with blood lead concentrations lower 2.2 μg/dL, and in those with BLLs ≥ 2.2 μg/dL (Figure 1). No significant association was observed between ALAD activity and BLLs for women with BLLs < 2.2 μg/dL. However, the results demonstrated a significant negative correlation (r = −0.413; $p < 0.01$) for women with BLLs ≥ 2.2 μg/dL.



(**A**) (**B**)

Figure 1. Linear regression between blood lead levels and δ-ALAD activity for women with BLLs < 2.2 μg/dL (**A**); and for thus with BLLs ≥ 2.2 μg/dL (**B**). The linear equation, correlation coefficient and *p* value are shown in the plot.

Table 3. Change in demographic characteristics, hemoglobin and ALAD activity by quartiles of blood lead levels.

Variables	First Quartile	Second Quartile	Third Quartile	Fourth Quartile	*p* *
n	160	158	158	157	
BLLs (μg/dL)	<1.09	1.09–1.61	1.62–2.19	>2.19	
Age, years	22.50 ± 6.84	23.60 ± 6.13	23.10 ± 6.08	23.20 ± 6.36	0.696
Education (years)	10.10 ± 2.70	10.18 ± 2.73	9.81 ± 2.60	10.05 ± 2.72	0.637
Gestational age (weeks)	13.69 ± 4.98	13.47 ± 4.73	13.71 ± 4.94	12.86 ± 4.79	0.375
Body mass index (kg/m^2)	24.90 ± 5.31	25.92 ± 5.39	26.01 ± 5.27	25.58 ± 4.98	0.254
Income per capita (USD per month)	98.41 ± 76.47	96.10 ± 100.04	95.99 ± 71.45	108.07 ± 106.64	0.614
Hemoglobin (g/dL)	12.88 ± 1.13	12.93 ± 1.20	12.95 ± 1.04	13.23 ± 1.64	0.070
ALAD activity, U/L	51.51 ± 21.82	59.10 ± 22.18	61.02 ± 19.10	58.82 ± 20.14	0.000

Note: * *p*-value was calculated from one-way ANOVA.

Table 4. Pearson correlations of blood lead levels with hemoglobin and ALAD activity by quartiles of blood lead levels.

Quartile of BLLs	Hemoglobin	ALAD Activity
First	0.027	−0.013
Second	−0.042	−0.043
Third	0.076	0.116
Fourth	−0.087	−0.413 **
All subjects	0.017	−0.113 **

Note: ** = Statistically significant correlation ($p < 0.01$).

To deepen the exploration of the relationship between blood lead concentration and ALAD activity in women with BLLs ≥ 2.2 μg/dL, multiple linear regression was applied (Table 5). Blood lead levels were inversely associated with ALAD activity ($p < 0.001$). However, no significant associations were found for age, educational level, gestational age, body mass index and hemoglobin. The model represents 21.9% of the predictive capability.

Table 5. Multiple linear regression model for ALAD activity in women with BLLs ≥ 2.2 μg/dL (n = 142).

Variable	Coefficient β	Standard Error	*p*-Value
Age, years	0.239	0.261	0.361
Educational level, years	0.689	0.578	0.235
Gestational age, weeks	0.202	0.339	0.553
Body mass index (kg/m^2)	−0.443	0.338	0.192
Hemoglobin (g/dL)	1.841	0.958	0.057
Blood lead levels (μg/dL)	−1.961	0.404	<0.001

Note: $R^2 = 0.219$.

4. Discussion

The mean blood lead concentration of 2.09 ± 2.34 μg/dL reported here is lower than those observed in other studies carried out in Mexican population. In Mexico City, Borja-Aburto found blood lead concentrations of 12.03 μg/dL in pregnant women who suffered spontaneous abortion and 10.09 μg/dL in a control group [4]. Another study of blood lead levels in pregnant women from Mexico City reported a mean blood lead concentration of 6.24 g/dL [27]. In a previous study carried out by our research group in pregnant women from Durango, Mexico, a mean blood lead level of 2.79 ± 2.14 μg/dL was observed, and 26 women (8.7%) had BLLs above the CDC recommended level of 5 μg/dL [24]. In the present research, also 26 women had levels of lead in blood above 5 μg/dL, but they represent 4.1% of the studied population.

Some authors have suggested that lead intoxication is characterized by high blood lead concentration and low ALAD activity [27,28]. For that reason, some researchers have recommended use of ALAD inhibition as an indicator of lead intoxication [12,21,29]. In our study, ALAD activity was significantly lower in women with BLLs $\geq$ 5 μg/dL compared with those with BLLs below 5 μg/dL. This finding is in an agreement with earlier published data. Similar results were observed in urban male adolescents from Lucknow, India [12], in children with neurological diseases from India [16], in lead workers from Taiwan [29], and in children from Southern Brazil [22].

Chiu et al. reported an inverse association between blood lead and ALAD activity when they compared lead workers from Taiwan with a control group (blood lead levels 19.5 $\pm$ 14.7 μg/dL and 2.9 $\pm$ 1.9 μg/dL, respectively) [29]. They concluded that the possible threshold value of blood lead for ALAD activity is around 10 μg/dL, and thus, ALAD activity may be usedas a biomarker for evaluation of lead toxicity in humans. Similar results were reported by Fecsa et al.; who analyzed lead dose-dependent effects for 18 lead exposed individuals and 12 normal volunteers [21]. Jasim et al. also reported a decrease of ALAD activity in battery manufacturing factory workers compared to non-exposed group; furthermore, this decrease became even more evident with increased duration of exposure [28]. The levels of lead in blood were 13.15 μg/dL in the control group, and more than 34.3 μg/dL in the exposed workers, respectively. In India, children residing in urban zones showed a negative correlation ($p < 0.001$) between blood lead levels (mean 11.8 $\pm$ 11.96 μg/dL) and ALAD activity [30].

Recent findings have suggested that ALAD inhibition may occur at low levels of lead in blood. Ahamed et al. reported a significant negative correlation between blood lead levels and ALAD activity in children with blood lead concentration lower than 10 μg/dL [15]. Moreover, Sakai and Morita considered that the threshold value of blood lead for ALAD inhibition is around 5 μg/dL [23]. Nevertheless, Martínez et al. did not find inhibition of enzymatic ALAD activity in children from Argentina, with mean blood lead of 2.58 $\pm$ 0.30 μg/dL [13].

Blood lead levels in our study were lower than in some prior studies on blood lead and ALAD activity [12,13,15,22,23,29,30]. Nevertheless, we observed a significant association between blood lead and ALAD activity at blood lead levels of 2.2 μg/dL, well below the CDC recommended level of 5 μg/dL for children and pregnant women [31]. To our knowledge, a similar result has not yet been reported in the literature.

It is well established that ALAD inhibition results in an increase of δ-ALA levels in blood, which can intensify oxidative stress and release iron from proteins such as ferritin [32]. For that reason, some authors have considered that decrease in ALAD activity has the potential to be used as an indicator of oxidative stress [32–34]. On the other hand, pregnancy is a condition that increases susceptibility to oxidative stress because of the mitochondria-rich placenta. During pregnancy, lipid peroxidation increases due to mitochondrial activity and hormone synthesis in placenta. Iron, which is abundant in the placenta, is important in the production of free radicals, and subjects the fetus to oxidative stress [35].

Importantly, our results also show that a small percent of pregnant women have blood lead concentrations above 5 μg/dL. Similar results were reported in a previous study carried out in Durango, Mexico [36]. A study conducted in Argentina, Mexico and Uruguay estimated 316,703 individuals in these countries are at risk of lead exposure, approximately 0.19% of the total population of all three countries. Of this population, 80,021 were women at childbearing age [37].

Researchers have documented that women with BLLs between 5–10 μg/dL have more probability of having a miscarriage compared to those with BLLs below 5 μg/dL [4]. It is thus necessary to identify and reduce the sources of exposure for these women. Recent research suggested a low threshold for the effect of maternal blood lead on birth outcomes, and recommended that exposure to lead during pregnancy should be kept as low as possible to minimize adverse outcomes [38]. Therefore, the growing evidence regarding the association between low levels of lead in blood and adverse pregnancy outcomes should be taken into account in the development of prevention politics.

We recognized some limitations in our study. In Figure 1 samples with blood lead between 5 and 10 μg/dL show quite a dispersion, but even in this segment the correlation is negative. In contrast, samples with blood lead below 2.2 μg/dL showed a slight increase of ALAD activity. It is well established that ALAD activity is specifically inhibited by lead at concentrations between 5 and 50 μg/dL [9]. In spite of this, significant correlations were observed only in the fourth quartile (BLL >2.19 μg/dL). In the other hand, we did not evaluate some biomarkers of oxidative stress that may be associated with blood lead [39], which could have resulted in uncontrolled confounding. Alcohol consumption may affect ALAD activity, but it was not considered because only a few women recognized they had this habit. Nonetheless, to our knowledge, this is the first study which has analyzed the relationship between blood lead levels and ALAD activity in Mexican pregnant women. Moreover, in the revised literature, there is no such data evaluating the effect of lead exposure on enzymatic ALAD activity in pregnant women, who constitute one of the most vulnerable sections of the population.

5. Conclusions

In summary, the results of our study suggest that even very low lead exposure may cause a decrease of ALAD activity, at least in pregnant women. We propose that ALAD inhibition may occur at very low levels of lead in blood due to lead exposure and pregnancy conditions.

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REVIEW
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Implications of DNA Methylation in Parkinson's Disease

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It has been 200 years since Parkinson's disease (PD) was first described, yet many aspects of its etiopathogenesis remain unclear. PD is a progressive and complex neurodegenerative disorder caused by genetic and environmental factors including aging, nutrition, pesticides and exposure to heavy metals. DNA methylation may be altered in response to some of these factors; therefore, it is proposed that epigenetic mechanisms, particularly DNA methylation, can have a fundamental role in gene–environment interactions that are related with PD. Epigenetic changes in PD-associated genes are now widely studied in different populations, to discover the mechanisms that contribute to disease development and identify novel biomarkers for early diagnosis and future pharmacological treatment. While initial studies sought to find associations between promoter DNA methylation and the regulation of associated genes in PD brain tissue, more recent studies have described concordant DNA methylation patterns between blood and brain tissue DNA. These data justify the use of peripheral blood samples instead of brain tissue for epigenetic studies. Here, we summarize the current data about DNA methylation changes in PD and discuss the potential of DNA methylation as a potential biomarker for PD. Additionally, we discuss environmental and nutritional factors that have been implicated in DNA methylation. Although the search for significant DNA methylation changes and gene expression analyses of PD-associated genes have yielded inconsistent and contradictory results, epigenetic modifications remain under investigation for their potential to reveal the link between environmental risk factors and the development of PD.

Keywords: 5-methylcytosine, DNA methylation, epigenetics, folate, alpha-synuclein, neurodegeneration, Parkinson disease

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INTRODUCTION

Parkinson's disease (PD) is the second most common chronic neurodegenerative disease in the elderly population. The motor symptoms that characterize PD are bradykinesia, tremor, rigidity, and postural instability, together with non-motor symptoms such as depression, anxiety, sleep disorders and cognitive dysfunction. These symptoms lead to severe impairment of the quality of life for the PD patient (Frucht, 2004). Pathological analyses of *post-mortem* brains have shown

Lewy bodies, which are abnormal protein aggregates found within nerve cells, and a progressive loss of *substantia nigra* dopamine neurons (Iacono et al., 2015). Among the molecular mechanisms suggested to cause PD are cellular oxidative stress and autophagy. Pesticide exposure, use of well water, heavy metal exposure, and industrialization are some of the environmental factors that contribute to the development of PD (Willis et al., 2010a,b).

Over recent years, extensive genetic screening of PD families has aimed at identifying mutations associated with the disease that would give a deeper insight into the molecular mechanisms underlying the PD pathology. Genetic studies identified several genomic risk *loci* associated with familial PD, such as *PARK1-15* and other related genes (Masliah et al., 2000; Cheon et al., 2012). Additionally, other genes, including *LRRK2*, *SNCA*, *MAPT,* and *GBA*, have been associated with sporadic PD (Coppede, 2012). Even though there is evidence that PD can be caused sporadically by familial genetic mutations (causal mutations), such as in *alpha-synuclein* (*SNCA*) or *Parkin*, it is more likely that in most patients the disease develops as a consequence of the combination of mutations in multiple PD-associated genes and environmental risk factors.

In addition to the genetic component involved in the development of many disorders (acquired mutations in one gene or a group of genes), epigenetic mechanisms have been found to contribute significantly to their development. Epigenetic factors are chemical modifications of chromatin or its regulatory proteins that do not change the underlying genomic sequence. These modifications can modulate gene expression, allowing differentiation into different cellular phenotypes by driving tissue-specific expression patterns. These changes include DNA methylation, post-translational modifications of histones, chromatin remodeling, as well as small and long non-coding RNAs (Turner, 2007).

Epigenetic regulation of biological processes is known to be essential during embryonic development, early brain programming, neurogenesis and brain plasticity (Yao et al., 2016). Therefore, it is not surprising that epigenetic deregulation can be critical for the onset of various neurodegenerative diseases, such as PD (Ammal Kaidery et al., 2013). Previously, a comprehensive genomic study identified several PD risk *loci* in cerebellum and frontal cortex of PD brains, including *PARK16*, *GPNMB*, and *STX1B* genes, that were associated with differential DNA methylation at proximal CpG sites (International Parkinson's Disease Genomics Consortium (IPDGC) and Wellcome Trust Case Control Consortium 2 (WTCCC2), 2011).

Importantly, as there is currently no animal model that mimics PD; human brain is still the model used to study epigenetic changes. However, as PD brain tissue is predominantly analyzed *post-mortem*, this data cannot provide information about disease-progressive alterations, the extent of variations induced by previous therapeutic treatments and the occurrence of potential PD biomarkers. Access to brain samples for research is limited, so the focus has been on finding a more easily accessible tissue, such as peripheral blood, as a surrogate for brain tissue. For this purpose, a genome-wide study examined DNA methylation changes in PD patients by collecting fresh *post-mortem* brain and blood samples from PD patients and age-matched healthy subjects (Masliah et al., 2013). This comparison revealed that both tissues exhibited highly similar global DNA methylation patterns. Accordingly, Masliah et al. (2013) identified groups of genes with either increased or decreased DNA methylation in both PD brain and blood samples. Importantly, analysis of DNA methylation profiles of blood clearly distinguishes PD patients from healthy subjects or subjects with other disorders. These results suggest that, firstly, peripheral blood may be a valid surrogate for brain tissue samples, and secondly, epigenetic changes could potentially serve as biomarkers for the diagnosis of PD. Early biomarkers could improve the prognosis of PD by facilitating the initiation of rational treatment before significant neurological damage takes place. Here, we discuss the current evidence for DNA methylation changes in PD, including the involvement of nutrition and environmental factors.

THE ROLE OF DNA METHYLATION IN DISEASE AND AGING

DNA methylation is the most studied epigenetic modification, one that has been investigated in almost all pathologies. In mammals, DNA methylation takes place predominantly in the context of CpG dinucleotides (Ehrlich and Wang, 1981). While overall the genome is widely depleted of CpGs, CpG islands are regions of high CpG content (Takai and Jones, 2002; Wu et al., 2010). CpG islands are characteristic for more than 60% of all promoters of protein-coding genes. Whereas on a genome-wide level up to 70–80% of all CpG sites are methylated, CpG islands are mostly devoid of DNA methylation (Bird, 2002; Edwards et al., 2010). Adjacent to a CpG island, 2 kilo base pairs (kb) up- and down-stream, are its so-called CpG shores (Irizarry et al., 2009). The presence of DNA methylation, at least at gene promoters and regions of repetitive sequences, is linked to chromatin silencing (Hsieh, 1994; Siegfried et al., 1999). Two principal mechanisms, which are not mutually exclusive, are thought to explain the repressive effect of DNA methylation on gene repression (Bird and Wolffe, 1999; Klose and Bird, 2006). First: DNA methylation interferes with the recognition of transcription factor (TF) binding sites and thereby impairs gene activation (Domcke et al., 2015). Second: DNA methylation is recognized by specific Methyl-CpG binding proteins, such as MeCP2, that recruit co-repressor protein complexes and thereby mediate silencing (Nan et al., 1998). Aberrant methylation patterns at CpG islands and shores have been linked to human disease, including multiple cancers (Ohm et al., 2007; Irizarry et al., 2009; Berman et al., 2011; Manjegowda et al., 2017).

In developing embryos and germ cells, DNA methylation patterns are first established by *de novo* DNA methyltransferases (DNMTs), DNMT3A and DNMT3B. After this, DNA methylation is maintained during DNA replication by DNMT1, which localizes to the replication fork during S-phase where it binds to hemimethylated CpGs (Jones and Liang, 2009; Jung et al., 2017). In 2009, ten-eleven translocation (TET) enzymes were identified that can reverse DNA methylation, by oxidization of 5-methyl cytosine (5mC) to 5-hydroxymethyl

cytosine (5hmC) (Tahiliani et al., 2009; Pastor et al., 2013). 5hmC can be lost passively by dilution during replication or be actively removed by subsequent oxidative reactions catalyzed by TET proteins that result in the formation of 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) as intermediates (Ito et al., 2011). Finally, the thymine DNA glycosylase (TDG)-mediated base excision repair (BER) replaces the methylated site by an unmodified cytosine (He et al., 2011; Kohli and Zhang, 2013).

Importantly, it appears that the 5hmC modification is not only an intermediate of 5mC demethylation, but has been considered to be an epigenetic mark in itself. Although the exact role of 5hmC is still being studied intensively, it seems to have a distinct function from that of 5mC. Particularly in neuronal cells, DNA hydroxymethylation was found to be enriched in gene bodies of actively transcribed genes (Mellen et al., 2012; Hahn et al., 2013). Given the relative abundance of DNA hydroxymethylation in the brain and its apparent role in normal brain maturation and memory formation (Szulwach et al., 2011; Kaas et al., 2013; Lister et al., 2013; Kinde et al., 2015), it has been implicated in the onset and progression of several neurodegenerative disorders (Villar-Menendez et al., 2013; Wang et al., 2013; Condliffe et al., 2014; Coppieters et al., 2014). Although not as widespread as 5mC and 5hmC, there is emerging evidence that cytosine methylation also exists outside of the sequence context of CpG sites (non-CpG methylation: CpA, CpT, and CpC) and appears to be most common in embryonic stem cells (Lister et al., 2009) and adult brain tissue (Varley et al., 2013; Guo et al., 2014). Non-CpG methylation occurs postnatally during the primary phase of neuronal maturation and may play a role in transcriptional repression (Lister et al., 2013; Guo et al., 2014). However, as it is technically challenging to target this modification *in vivo* without altering CpG methylation in the process, extensive research is still required to elucidate the distinct biological function of non-CpG methylation.

Genetically, aging is characterized by distinct alterations that take place at the chromatin level. These include telomere shortening, increased genome instability and changes of epigenetic signatures, such as DNA methylation patterns (Lopez-Otin et al., 2013). Age-related remodeling of DNA methylation comprises events of both hypo- and hypermethylation (Maegawa et al., 2010; Jung and Pfeifer, 2015). DNA hypomethylation happens globally at CpG sites outside of CpG islands (Christensen et al., 2009; Heyn et al., 2012; Day et al., 2013), while DNA hypermethylation affects mostly CpG islands in promoters of genes, which are frequently involved in development and differentiation (Christensen et al., 2009; Rakyan et al., 2010). Therefore, over time the accumulation of epimutations, which are heritable changes of gene activity mediated by epigenetic alterations, are believed to contribute to genomic instability just as genetic mutations do.

Interestingly, the methylation states at specific CpG *loci* can be consulted as epigenetic biomarkers to reliably predict the human chronological age (Horvath, 2013; Weidner et al., 2014). Moreover, twin studies determined that in each human, the age-dependent aggregation of distinct epigenetic changes, termed 'epigenetic drift,' is thought to be influenced predominantly by environmental factors (Fraga et al., 2005; Tan Q. et al., 2016). The individual differences in exposure to these factors are suspected to contribute to variation in disease susceptibility, onset, progression, etiopathology, treatment response and disease outcome. Taken together, changes in DNA methylation patterns and their effects on chromatin and gene expression appear to add increasingly to our understanding of age-related diseases, including PD. In the following, we will summarize and discuss the current evidence of DNA methylation changes at candidate genes that could be related to the development of PD.

DNA METHYLATION AND PD: ANALYSIS OF THE *SNCA* GENE

The *SNCA*p.Ala53Thr mutation, described in 1997, was the first genetic cause of PD identified (Polymeropoulos et al., 1997). This missense mutation provided the first link between *SNCA* and familial PD after its identification in a family from Southern Italy. The respective gene product, the SNCA protein, was discovered almost simultaneously (Spillantini et al., 1997). At the molecular level, SNCA aggregation contributes majorly to the formation of Lewy bodies, a hallmark of PD pathology. In addition to genetic mutations, also *SNCA locus* amplifications (duplications, triplications) have been found as a cause of familial PD (Singleton et al., 2003; Chartier-Harlin et al., 2004). *SNC*A point mutations, as well as gene multiplications and overexpression, are all thought to play a causal role in the formation of Lewy bodies (Narhi et al., 1999; Masliah et al., 2000). That *SNCA* gene dosage is critical for the development of PD, was further supported by mouse models with neuronal expression of wild-type *SNCA* (Masliah et al., 2000; Janezic et al., 2013). These transgenic mice revealed PD-like loss of dopaminergic neurons, protein aggregate formation, and motor impairments.

As gene dosage can be changed not only by gene amplification, but also by gene regulation, DNA methylation was considered as a potential mechanism that could be involved in the deregulation of *SNCA* in the case of PD. Accordingly, sequence analysis of the promoter region of the *SNCA* gene led to the identification of two CpG islands (Matsumoto et al., 2010). The first, CpG-1 is located in the first exon but does not overlap with the coding region of *SNCA*, and the second, CpG-2 is located in the first intron. In luciferase reporter assays the promoter activity of sequences containing CpG-2 was indeed strongly reduced by *in vitro* DNA methylation prior to cell transfection (Jowaed et al., 2010; Matsumoto et al., 2010). Furthermore, treatment of SK-N-SH cells with a DNA methylation inhibitor resulted in a reduction of CpG-2 methylation and a significant increase of *SNCA* mRNA and protein levels (Jowaed et al., 2010). These data supported the idea that DNA methylation at least at the intronic CpG-2 island could control *SNCA* gene activity. In fact, several studies analyzing the DNA methylation levels in samples of PD patients compared to controls confirmed a hypomethylation of intron 1 that coincides with the second *SNCA* CpG island. Using brain samples a significant demethylation of intron 1 was reported in the *substantia nigra pars compacta* (SnPC) of PD patients which could explain increased *SNCA* expression (Jowaed et al., 2010; Matsumoto et al., 2010). Taking into account the

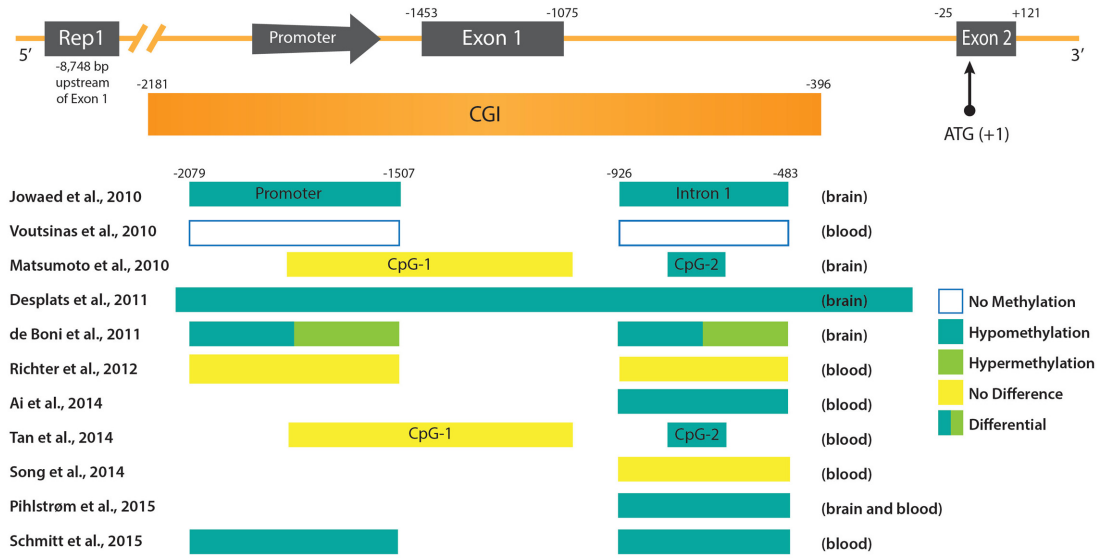

FIGURE 1 | DNA methylation patterns in PD at the promoter region of the *SNCA* gene. **(Upper)** The promoter region and 5′ end of the *SNCA* gene containing the core promoter and the two first exons of *SNCA* (Exon 1 and Exon 2) are depicted. Exon 2 contains the ATG start codon of the open reading frame (ORF). Upstream of the promoter the location of the polymorphic microsatellite sequence Rep1 is shown. Below the scheme of the *SNCA* gene the CpG-rich region is depicted as an CpG island (CGI). Positions of regulatory sequences (promoter and CGIs), as well as exons and introns are given at the top in reference to the ATG start codon. **(Lower)** Regulatory sequences and intronic regions analyzed in various studies (see text for more detail) are depicted as boxes. The references are given on the left, and the DNA methylation status and source of PD samples on the right.

observation that DNA methylation patterns between blood and brain tissue show a strong correlation (Masliah et al., 2013), more recent studies analyzed peripheral blood samples of PD subjects instead of or in addition to *post-mortem* brain tissue. In agreement with the assumption that DNA methylation profiles in the brain could be potentially mirrored in blood cells, a recent study found *SNCA* promoter hypomethylation in both *post-mortem* cortex and peripheral blood samples (Pihlstrom et al., 2015). Another study reported hypomethylation of *SNCA* intron 1 in peripheral blood mononuclear cells of 100 sporadic PD subjects (Ai et al., 2014). In 2015, the largest study carried out to date analyzing 490 peripheral blood samples of patients with sporadic PD, also revealed hypomethylation of *SNCA* intron 1. In contrast, *SNCA* methylation was found to be increased in PD patients who received higher L-dopa dosage. Accordingly, L-dopa led to a specific increment of DNA methylation of *SNCA* intron 1 in mononuclear cell cultures. Interestingly, the detection of DNMT1 in *post-mortem* brain tissue of PD patients and in *SNCA* transgenic mice uncovered that the amount of enzyme was strongly reduced in the nuclear fraction of neuronal cells (Desplats et al., 2011). Thus, sequestration of DNMT1 in the cytosol could explain the global, as well as the *SNCA* gene-specific, PD-dependent DNA hypomethylation, mechanistically.

In recent years, meta-analyses of genome-wide association studies (GWAS) on single nucleotide polymorphism (SNP) data of large PD case-control cohorts were conducted (Nalls et al., 2011, 2014; Sharma et al., 2012). These studies identified risk *loci* in both genes, previously not linked to PD pathology, and known key players, such as *SNCA*. Thereby, obtained results substantiated that there is a major genetic component contributing to the susceptibility to PD. But additionally, inter-individual genetic variants can frequently be associated with DNA methylation differences at distinct CpG sites and are defined in statistical analyses as methylation quantitative trait *loci* (mQTLs). Two recent studies investigated the relationship between genetic variation and CpG methylation in the human brain (Gibbs et al., 2010; Zhang et al., 2010). In case of the *SNCA* gene, three independent studies noted that the genotype SNP rs3756063 showed a significant correlation with the DNA methylation state of *SNCA* intron 1 both in brain and blood PD samples (Pihlstrom et al., 2015; Schmitt et al., 2015; Wei et al., 2016). However, it should be noted that an association between rs3756063 and the *SNCA* mRNA expression could not be found (Pihlstrom et al., 2015; Wei et al., 2016). Furthermore, another association could be established between the *SNCA* DNA methylation levels and the Rep1 polymorphism (Ai et al., 2014). In contrast to rs3756063, Rep1 is a complex microsatellite repeat polymorphism located approximately 10 kb upstream of the *SNCA* transcription start site (**Figure 1**). Its longest 263 bp allele has previously been associated with sporadic PD (Maraganore et al., 2006). In agreement with an elevated PD risk, genotypes carrying the 263 bp allele showed the strongest *SNCA* intron 1 hypomethylation (Ai et al., 2014). Experiments in transgenic mice suggested a *cis*-regulatory effect of the Rep1-length regulating *SNCA* transcription, whereby homozygosity of the expanded 263 bp allele correlated with the highest gene expression (Cronin et al., 2009). Controversially, recent data obtained by the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 technique, to edit the genome Rep1 *locus* in human embryonic stem cell-derived neurons, contradict

the enhancer function of the repeat sequence and do not detect a correlation between *SNCA* expression and Rep1-length (Soldner et al., 2016). Likewise, the interdependency between DNA methylation alterations in the *SNCA locus* and genetic variants, is not understood mechanistically, neither in case of Rep1 nor rs3756063. Genetic variation does not only impact DNA methylation, but also dictates differences in binding of TFs in individuals on a genome-wide level (Kasowski et al., 2010). Therefore, it will be a great challenge to unravel the molecular mechanisms behind these associations, as they are expected to be connected with each other in a complicated network.

Although the studies cited above suggest DNA hypomethylation of the *SNCA* promoter region in PD patients, discrepancies with other findings exist. For example, one study revealed variations of DNA methylation levels at the *SNCA* gene in different brain regions. Both hypomethylation, as well as hypermethylation, were detected in various Lewy body disease/PD stages in both the promoter region and intron 1 (de Boni et al., 2011). Another recent study found no intron 1 hypomethylation of *SNCA* in a limited number of PD patients (Guhathakurta et al., 2017). Moreover, the analysis of blood samples from 43 PD subjects provided no evidence for DNA methylation changes within the *SNCA* promoter region (Richter et al., 2012), just as another study comprising blood leukocyte samples of 50 PD patients (Song et al., 2014). However, it should be noted, that in the latter two studies (Richter et al., 2012; Song et al., 2014) 10 times less subjects participated compared to the analysis of Schmitt et al. (2015) that detected differential DNA methylation at the *SNCA* gene.

In light of the inconsistencies concerning the relevance of DNA hypomethylation at the *SNCA* intron 1 in association with PD, additional studies will be needed to resolve these doubts. Also, even if *SNCA* intron 1 hypomethylation can be consistently confirmed in PD, similar DNA patterns were also found in both Dementia with Lewy Bodies (DLB) (Funahashi et al., 2017) and AD (Yoshino et al., 2016). Therefore, it is conceivable that this DNA methylation change would not serve as a specific biomarker for PD, but a more general one for all Lewy body pathologies.

DNA METHYLATION PATTERNS AT PD-ASSOCIATED GENES

In the following section, we will give an overview of what is known so far about the epigenetic signatures of DNA methylation at genes that were found to be associated with the development of PD (compare **Table 1**).

Due to its relation to *SNCA* and its abundance in neurofibrillary lesions of patients with AD, the *beta-synuclein* (*SNCB*) gene has also been considered as a possible player in PD. Interestingly, SNCB inhibits the generation of SNCA fibril aggregation *in vitro* (Park et al., 2003) and therefore, may play a neuroprotective role (Vigneswara et al., 2013). Furthermore, *SNCB* and *SNCA* have similar expression levels in nervous system tissue samples (Maroteaux et al., 1988). Moreover, SNCA and SNCB associate *in vitro* whereby SNCB may protect SNCA from aging-related protein damage (Vigneswara et al., 2013). However, to date, the specific role SNCB plays in PD has not been elucidated. A DNA methylation study carried out in PD samples to examine the *SNCB* gene found its promoter to be unmethylated in *post-mortem* brain from PD and PD-Dementia samples. Additionally, bisulfite sequencing of the *SNCB* promoter in four pure diffuse Lewy body pathology cases did not reveal methylated cytosines along the CpG island (Beyer et al., 2010).

Post-translational citrullination (deimination) is mediated by peptidyl arginine aminases (PADs) and has been implicated as an unusual pathological trait in neurodegeneration and inflammatory responses in multiple sclerosis, AD and prion diseases (Jang et al., 2013). Alterations in the expression of these proteins have also been seen in *post-mortem* samples taken from different brain areas of PD subjects (Nicholas, 2011). In thymus samples from multiple sclerosis patients, the promoter of *PAD2* (peptidyl arginine deaminase type II) was reported to be hypomethylated (Sokratous et al., 2016). In contrast, white matter from PD, AD, or Huntington disease patients showed that *PAD2* was not hypomethylated (Mastronardi et al., 2007). DNA methylation analysis of the tumor necrosis factor alpha (TNF-alpha) gene, another PD-associated gene, showed a significantly lower methylation level comparing DNA from the SnPC to DNA from brain cortex. However, this difference could not be linked to PD as it was observed in both PD subjects and controls (Pieper et al., 2008). In another study, the *UCHL1* promoter from *post-mortem* frontal cortex samples was analyzed, and no differences in the percentage of CpG methylation between PD cases and controls were found (Barrachina and Ferrer, 2009).

Behrens et al. analyzed the *ATP13A2* promoter region from four PD subjects with Kufor-Rakeb syndrome, a rare Type 9 juvenile PD that is linked to a mutation in the *ATP13A2* gene (Behrens et al., 2010). No significant correlation between DNA methylation changes of the hypomethylated promoter and Kufor-Rakeb syndrome juvenile PD progression was found. Another study considered the known association of *Parkin* (*PARK2*) gene mutations with autosomal recessive juvenile PD. Samples from 17 PD subjects with heterozygous *Parkin* mutations, as well as 17 PD subjects without *Parkin* mutations, were compared to samples from 10 normal subjects. No significant differences in DNA methylation at CpG sites among these three groups were found, suggesting that a DNA methylation-related mechanism involving the *Parkin* gene was unlikely to play a role in the pathogenesis and development of this type of PD (Cai et al., 2011). A recent study compared the DNA methylation status of the *PARK2* promoter region in 5 *post-mortem* brain samples taken from *substantia nigra*, cerebellum, and occipital cortex (De Mena et al., 2013). In agreement, with previous results (Cai et al., 2011) no differential DNA methylation of *PARK2* was seen (De Mena et al., 2013).

The expression of clock genes, which are components of the circadian clock, is altered in leukocytes from patients with PD (Cai et al., 2010). With this in mind, a study was recently carried out in which DNA methylation status of the clock genes *PER1*, *PER2*, *CRY1*, *CRY2*, *Clock*, *NPAS2*, and *BMAL1* was measured in genomic DNA isolated from blood samples

TABLE 1 | DNA methylation status of PD associated genes.

Gene	Alias	Location	DNA methylation	Reference
PAD2	Peptidyl arginine deiminase 2	1p36.13	No difference	Mastronardi et al., 2007
ATP13A2	*PARK9*	1p36.13	No difference	Behrens et al., 2010
DJ-1	*PARK7*	1p36.23	No methylation	Tan Y. et al., 2016
NPAS2	Neuronal PAS2	2q11.2	Hypomethylation	Lin et al., 2012
UCHL1	Ubiquitin C-terminal hydrolase L1	4p13	No difference	Barrachina and Ferrer, 2009
PGC1-α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha	4p15.2	Hypermethylation	Su et al., 2015
TNF-α	Tumor necrosis factor-alpha	6p21.33	No difference	Pieper et al., 2008
PARK2	Parkinson juvenile disease protein 2, *Parkin*	6q26	No difference	Cai et al., 2011
			No difference	De Mena et al., 2013
CYP2E1	Cytochrome P450-J	10q26.3	Hypomethylation	Kaut et al., 2012
NOS2	Nitric oxide synthase 2	17q11.2	Hypomethylation	Searles Nielsen et al., 2015
MAPT	Microtubule associated protein Tau	17q21.31	Differential methylation	Coupland et al., 2014
FANCC/TNKS2	Fanconi anemia group C protein/tankyrase 2	9q22.32/10q23.32	Differential methylation	Moore et al., 2014
PARK16/GPNMB/STX1B	*PARK16*/glycoprotein Nmb/syntaxin 1B	1q32/7p15.3/16p11.2	Differential methylation	International Parkinson's Disease Genomics Consortium [IPDGC], and Wellcome Trust Case Control Consortium 2 [WTCCC2]
Genome-wide	Top 30 differentially methylated genes: *KCTD5, VAV2, MOG, TRI M10, HLA-DQA1, ARHGEF10, GFPT 2, HLA-DRB5, TMEM9, MRI 1, MAPT, HLA-DRB6, LASS3, GSTTP 2, GSTTP.*		Hypermethylation	Masliah et al., 2013
	DNAJA3, JAKMIP 3, FRK, LRR C27, DMBX1, LGALS7, FOXK1, APBA1, MAGI2, SLC25A24, GSTT 1, MYOM2, MIR886, TUBA3E, TMCO3		Hypomethylation	

From left to right: Listed are genes, gene aliases, genomic locations (according to the latest GRCh38/hg38 assembly of the human genome available at the UCSC genome browser), DNA methylation status in PD and references.

of 206 PD subjects. DNA methylation was detectable in *CRY1* and *NPAS2* promoters whereas the remaining gene promoters analyzed were devoid of DNA methylation. Interestingly, DNA methylation frequency of the *NPAS2* promoter was significantly decreased in PD patients, suggesting that its promoter DNA methylation may contribute to the expression of clock genes in PD (Lin et al., 2012). This finding could be relevant, as sleep disturbance is a commonly reported early symptom of PD (Breen et al., 2014).

Another gene of interest for the analysis of epigenetic changes is the microtubule-associated protein tau (*MAPT*) gene, as a genetic association with PD has been noted in GWAS (Simon-Sanchez et al., 2009). When 28 *post-mortem* brain and 358 blood leukocyte samples were analyzed, higher DNA methylation in MAPT was detected in H1 haplotype versus H2 (Coupland et al., 2014). Notably, in previous studies the presence of the H1 haplotype was associated significantly with PD (Kwok et al., 2004; Zabetian et al., 2007; Refenes et al., 2009). Additionally, DNA hypermethylation of the MAPT gene was observed in the cerebellum, but not in putamen from PD subjects where the MAPT gene was hypomethylated as compared with controls (Coupland et al., 2014).

DNA hypermethylation of the peroxisome proliferator-activated receptor gamma coactivator-1 α (PGC-1α) promoter was reported in a sample of sporadic PD *substantia nigra* samples compared to 10 age-matched controls (Su et al., 2015). Recently, *PARK7* (*DJ-1*) DNA methylation was analyzed in peripheral blood leukocytes in PD subjects and controls. In contrast to the hypermethylated PGC-1α promoter (Su et al., 2015), they found the CpG-1 and CpG-2 islands of *PARK7* to be unmethylated in both PD and the negative control group (Tan Y. et al., 2016).

To detect further PD associated DNA methylation variations, an epigenome-wide association study was done to analyze DNA methylation patterns in putamen samples from *post-mortem* brain tissue of six PD patients. DNA methylation levels were quantitatively determined at 27,500 CpG sites representing 14,495 genes. This analysis revealed decreased DNA methylation at the cytochrome P450 2E1 (*CYP2E1*) gene, together with increased expression of the respective *CYP2E1* messenger RNA, suggesting that this cytochrome gene may contribute to PD susceptibility. In another epigenome-wide association study, conducted to reveal prioritized genes and pathways with statistically significant DNA methylation changes in PD, followed by a subsequent replication analysis

of top-ranked CpG sites, single CpG sites of *FANCC* and *TNKS2* showed significant differential methylation between PD cases and controls (Moore et al., 2014). In total, 20 unique genes were identified with a sizable difference in DNA methylation.

Despite the lack of conclusive evidence for the involvement of DNA methylation in the epigenetic regulation of many PD-associated genes, the search for other PD-associated genes and their DNA methylation status is ongoing. Undoubtedly, the significance and consistency of results of genomic DNA methylation in promoter regions of blood samples in comparison with brain samples need to be tested further. Unfortunately, findings from studies searching for PD-specific DNA methylation signatures at the *SNCA* gene and other PD-associated genes are still inconsistent concerning the clinical significance and specificity of DNA methylation changes in PD. Most importantly, experimental evidence that directly links DNA methylation changes in PD to the deregulation of these genes is still missing. Thus, the importance of differential DNA methylation for molecular mechanisms contributing to the development of PD remains to be investigated in future studies.

NUTRITIONAL FACTORS AND THEIR IMPLICATIONS FOR PD

DNA Methylation and Folate Deficiency

In recent years, several studies have attempted to pinpoint an interrelation between DNA methylation and folate. However, a significant association between DNA methylation levels and folate status could not always be consistently replicated (Waterland and Jirtle, 2003; Waterland et al., 2006; Steegers-Theunissen et al., 2009; Tobi et al., 2009; Shin et al., 2010). Apart from folate, many other nutrients are known to play key roles in one-carbon metabolism and DNA methylation. More accurate studies that analyze the contribution of other nutrients involved in DNA methylation and gene-diet interactions for PD risk are necessary.

The extent of DNA methylation in the cell is directly associated with the physiological level of SAM, the major methyl donor for DNA methylation, and SAH, the demethylation product of SAM and an inhibitor of DNA methyltransferases. The ratio SAM/SAH is interpreted as the methylation potential and is determined by the homocysteine concentration. The latter is considered a biomarker of folate deficiency, as it is dependent on 5-methyl tetrahydrofolate (THF) availability in the one-carbon metabolism. The physiologic levels of homocysteine and subsequent methylation potential are determined primarily by the dietary intakes of methionine, folate, B12 vitamin and other nutrients.

Importantly, elevated levels of homocysteine may have a toxic effect on dopaminergic neurons (de Lau et al., 2005; Gorgone et al., 2012). Consistently, higher homocysteine concentrations have been reported in PD patients compared to controls. Moreover, serum homocysteine levels predict the SAM/SAH ratio in plasma, and the concentration of SAH shows a significant correlation with markers of neurodegeneration (Amyloid Precursor Protein and SNCA). This evidence supports the use of total homocysteine and SAM/SAH ratio as biomarkers of the DNA methylation potential in patients with PD (Obeid et al., 2009). However, we still lack information about the direct effect of nutrient intake on genomic DNA methylation, especially regarding the combinatorial effects of nutrients with other factors, such as gene polymorphisms and/or therapeutic drugs.

One-Carbon Metabolism and Polymorphisms

The presence of SNPs in genes encoding enzymes and transporters involved in the folate metabolism, impair methyl group bioavailability and have been associated with altered blood concentrations of biochemical markers, including folate, vitamin B12 and homocysteine (Hazra et al., 2009; Tanaka et al., 2009; Liang et al., 2014). Importantly, some SNPs led to changes of homocysteine levels and were associated with differences in global DNA methylation levels (Wernimont et al., 2011). Other SNPs have also been associated with diseases, such as neural tube defects (Carter et al., 2011; Fisk Green et al., 2013; Ouyang et al., 2013; Liu et al., 2014) and different types of cancers (Curtin et al., 2007; Collin et al., 2009; Gibson et al., 2011; Levine et al., 2011; Metayer et al., 2011; Weiner et al., 2012).

The C677T variant (rs1801133), in the gene encoding the enzyme methylene-tetrahydrofolate reductase (MTHFR), is one of the most-studied SNPs occurring in components of the one-carbon metabolism. The base C677T substitution results in an amino acid change in the catalytic domain of the enzyme. This variation leads to a reduced protein stability and a 30% and 65% reduction of enzymatic activity in heterozygotes (CT) and homozygotes (TT), respectively (Rozen, 1997). Notably, the C677T variant has been previously reported to be associated with PD susceptibility (de Lau et al., 2005; Wu et al., 2013). A recent meta-analysis including data from fifteen studies (comprising 2690 PD cases and 8465 controls) did not find an appreciable difference in the general allelic frequency distribution of C677T between PD cases and controls (Zhu et al., 2015). However, in separate analyses that were stratified for ethnicity, a clear association was detected in Europeans (OR = 1.17), but not in Asians. Interestingly, this appears to be in line with the observation that the allelic frequency of the MTHFR C677T variant differs considerably between ethnic groups (Wilcken et al., 2003; Gueant-Rodriguez et al., 2006). Furthermore, this study confirmed that the T allele is an independent risk factor for increased homocysteine levels in PD patients (Zhu et al., 2015). In contrast, the results of a cohort study analyzing Chinese patients suggested that the A-T haplotype of A1298C, another common MTHFR variant, and C677T decreases the PD susceptibility (Yuan et al., 2016). The inconsistent findings for the association between C677T and PD may be explained by different genetic backgrounds, environmental factors or DNA methylation modulation.

OTHER NUTRITIONAL/ENVIRONMENTAL FACTORS AND DNA METHYLATION

Coffee Drinking

The risk for PD is ∼25% lower for coffee drinkers with a linear dose-response effect (Costa et al., 2010; Delamarre and Meissner, 2017). Caffeine is thought to act as an adenosine receptor antagonist, and to reduce inflammation and lipid-mediated oxidative stress (Farooqui and Farooqui, 2011; Kolahdouzan and Hamadeh, 2017).

Little is known whether DNA methylation changes can arise in response to distinct coffee consumption patterns. A recent study using blood tissue data of patients without PD found the methylation status of CpG sites located near genes previously linked to some familial forms of PD (*GBA*, *PARK2/Parkin*, and *PINK1*) associated with coffee consumption (Chuang et al., 2017). However, whether distinct DNA methylation levels at these CpG sites in coffee-drinkers are indeed protective against PD, remains to be further investigated.

Manganese

Manganese (Mn) is an essential element, but some industrial activities can result in exposure to high occupational and environmental Mn levels (Bowler et al., 2011, 2016). In the environment, Mn in drinking-water and foods may also contribute to toxic effects (ATSDR, 2012).

Exposure to excessive amounts of Mn may lead to adverse health outcomes, and evidence suggests that DNA methylation changes induced by Mn may play a relevant role. Regarding PD risk, gene activity of *PARK2* and *PINK1* was altered via DNA hypermethylation in dopaminergic human neuroblastoma SH-SY5Y cells upon Mn exposure (Tarale et al., 2016). Furthermore, mice exposed to $MnCl_2$ showed DNA hypo- and hypermethylation of different *loci* in *substantia nigra* (Yang et al., 2016). In human, the effects of Mn on parkinsonism via DNA methylation changes was assessed in welders' blood samples. Interestingly, subjects recently exposed to welding fume had lower *NOS2* gene DNA methylation than subjects retired from welding worksites. Also, an inverse association between duration of welding fume exposure and DNA methylation of a *NOS2* CpG site was observed (Searles Nielsen et al., 2015).

Endocrine Disruptors and Pesticides

It is proposed that other factors, such as endocrine disruptors or pesticide exposure, may play a role in modulating DNA methylation, although the evidence from studies with PD patients or animal models is still limited. Results from experimental, clinical, and epidemiological studies implicate exposure to endocrine disruptors with processes related to neurodegenerative diseases (Kajta and Wojtowicz, 2013; Preciados et al., 2016). Among these compounds, Bisphenol-A has been linked to lower levels of DNA methylation in cerebral cortex and hippocampus in mice (Kumar and Thakur, 2017). Several studies have shown an association with frequent pesticide exposure in men and late-onset PD (Delamarre and Meissner, 2017). Recent findings show that organochlorines exposure of hippocampal-primary cultures causes global hypomethylation of DNA (Wnuk et al., 2016).

Although research suggests that these and other environmental exposures can modify epigenetic signatures; important questions remain open. Therefore, studies in this field will provide new insights into PD pathologic processes, and consequently provide novel preventive and therapeutic intervention strategies.

CONCLUDING REMARKS AND PERSPECTIVES

Currently, there is a plethora of methods used for measuring DNA methylation (Kurdyukov and Bullock, 2016). To date the "gold standard" for the quantification of DNA methylation is still considered to be bisulfite sequencing. Nowadays this method is often used for genome-wide studies in combination with next-generation sequencing. However, the generation of bisulfite-converted DNA, and its subsequent use has often been described as technically challenging. Additionally, bisulfite conversion can lead to DNA fragmentation and can make amplification of long DNA regions difficult while resulting in chimeric products (Kurdyukov and Bullock, 2016). An easier method is needed that does not require bisulfite conversion, for example, an endonuclease digestion-based assay (historically the first technique utilized for studying DNA methylation), which can be applied at gene-specific *loci*, but is also compatible with whole genome methylation profiling. Determining a standardized method for quantifying DNA methylation at the same genomic regions of reported PD-associated genes would be ideal for clinical research. Another issue is finding a method that can efficiently detect 5hmC and distinguish it from 5mC, not only on a genome-wide level, but also at bp resolution. Techniques applied to analyze DNA methylation changes associated with PD have mostly not discriminated between 5mC and 5hmC. Particularly in the brain, it will be of great interest to unravel whether the latter DNA methylation mark exhibits disease-specific patterns that could serve as biomarkers. A recent study found an approximate two-fold increase of global DNA hydroxymethylation in the cerebellum of PD patients (Stöger et al., 2017). As this analysis lacks information about where these changes take place in the genome, further experiments will need to shed light on the cause, and examine whether elevated 5hmC levels contribute to PD onset or progression or PD is the reason for the aberrant hydroxymethylation. Approaches that can specifically detect 5hmC have been described, e.g., antibody-based techniques or oxidative bisulfite sequencing (Booth et al., 2012; Skvortsova et al., 2017). These efforts make it likely that methods that discriminate between 5mC and 5hmC will be on hand in the future.

For now, more epigenetic studies are required, particularly ones conducted in different populations, to expand the currently available database of DNA methylation in PD-associated genes. Importantly, a more accurate consensus needs to be reached on

the benefit of peripheral blood samples versus brain samples. For the DNA methylation status of a specific gene promoter, such as *SNCA* or other PD-associated genes to be authenticated as a reliable biomarker of PD status, a significant number of studies reporting consistent results will be needed. In this context, careful analysis of Levodopa treatment effects on *SNCA* DNA methylation offers the prospect that in the not-so-distant future a reliable DNA methylation biomarker in PD with high sensitivity and specificity will be available (Schmitt et al., 2015). With the growing interest in research on the interdependency between nutrition and epigenetics, in the future we will get a better understanding of what effects nutritional factors have on DNA methylation and what their involvement is in diseases like PD.

Epigenetic modifications may prove to be the missing link between environmental risk factors and the development of PD. Epigenetic variances between individuals could help us to explain the striking clinical differences observed in the age of onset and progression of sporadic PD, and may open the way for rational therapeutic intervention targeting DNA methylation modifications associated with this disorder.

AUTHOR CONTRIBUTIONS

EM-M, KM, AS-C, JS-P, PV-C, and OA-C wrote the manuscript.

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Research article

H1/H2 *MAPT* haplotype and Parkinson's disease in Mexican mestizo population

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ABSTRACT

Parkinson's disease (PD) is characterized by bradykinesia, resting tremor, rigidity and postural instability as well as early symptoms. Previous studies that evaluated the association between H1/H2 *MAPT* haplotype and PD were mostly conducted in European populations in which the H1 haplotype was a reported risk factor for PD. Despite those findings, some studies have suggested that the association may be ethnically dependent. Since studies conducted in Latin American population have been scarce, we genotyped the H1/H2 *MAPT* haplotype in Mexican mestizo population as part of a PD case-control study. DNA was extracted from peripheral blood leucocytes in 108 cases and 108 controls and detection of the H1/H2 haplotypes was achieved by determining the MAPT_238 bp deletion/insertion variant at intron 9 through end-point PCR followed by visual 3% agarose gel electrophoresis interpretation. We observed no-association between genotypes and PD risk [OR/CI (Odds ratio/95% Confidence Interval) of 1.60 (0.78–3.29) for H1/H2 genotype and 2.26 (0.20–25.78) for H2/H2]. No-association was maintained when stratifying our groups by central ($p = 0.27$) and northern regions ($p = 0.70$). Our data suggest that H1/H2 *MAPT* haplotype is not a risk factor to PD in our population.

1. Introduction

1.1. Pathological mechanisms

Parkinson's disease (PD) is the second most common neurodegenerative disease and is characterized by bradykinesia, resting tremor, rigidity and postural instability as well as early symptoms such as hyposmia, constipation and sleep disorders, among others [1]. Pathological mechanisms include the death of melanin and dopamine-producing neurons in the Substantia Nigra Pars Compacta (SnPC) and the loss of Neuromelanin (NM) as a post-mortem feature [2]. Reports also identified alpha-synuclein [3] and tau [4] aggregates in Lewy Bodies. While the presence of metal ions and mitochondrial reactive oxygen species (ROS) formation have also been demonstrated [5]. Genetic causes were found in familial and sporadic PD [6]. Microtubule Associated Protein Tau (*MAPT*) gene has been highly associated [7] and studies reported an over-representation of the MAPT H1 haplotype in various neurodegenerative disorders including progressive supranuclear palsy [8], Alzheimer's Disease (AD) [9], and PD [10].

1.2. MAPT H1/H2 structure

The *MAPT* locus contains two reported haplotypes: the directly oriented H1 and the inverted H2 [11–13]. The H1 and H2 haplotypes may be distinguished by identifying one or more of the eight reported single nucleotide polymorphisms (SNPs) in absolute linkage disequilibrium. Another characteristic of H2 is the presence of a 238 bp deletion within intron 9 (Fig. 1) [8].

1.3. MAPT H1/H2 associations

A copious amount of studies have been conducted to determine the possible association between H1/H2 haplotypes and PD with contradictory results. A recent meta-analysis determined that the *MAPT*_238 bp deletion/insertion might modulate the risk of PD [14]. Also, studies in Greek and Serbian populations reported association

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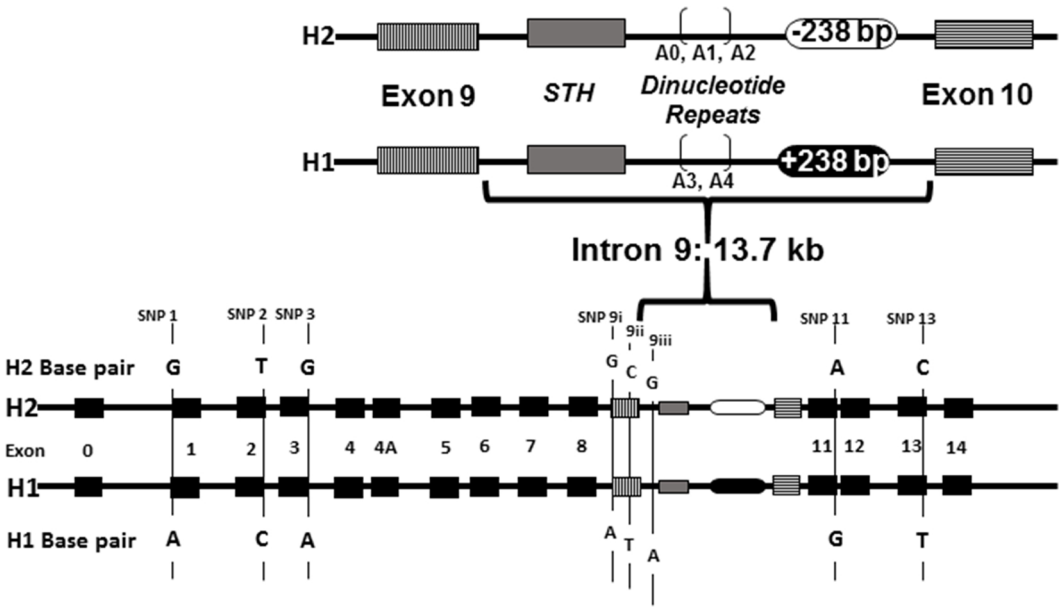

Fig. 1. Schematic representation of The *MAPT* region of interest. H1 and H2 contain 8 distinct SNPs (vertical lines with respective base pairs) and the MAPT_238 bp deletion (white oval)/insertion (black oval) variant at Intron 9. Black boxes indicate exons. Gray box indicates Saitohin (*STH*).

between H1 Haplotype and PD [15–17] and a report in UK Caucasian population found the H2 haplotype to be a protective factor [18]. However, reports in German, Indian, Greek and Finnish populations suggested that there is no correlation between H1/H2 haplotypes and PD [17,19,20]. The main goal of this work was to determine if there is an association between H1/H2 haplotypes and PD in a Mexican mestizo population.

2. Materials and methods

2.1. Information about participants

The subjects were recruited from three public hospitals. General Hospital Dr. Manuel Gea González in Mexico City (central region of Mexico), General Hospital 450 in Durango (northern region of Mexico) and General Hospital Santiago Ramón y Cajal in Durango (northern region of Mexico). PD was diagnosed using the UK Parkinson's Disease Society Brain Bank Diagnostic Criteria (UKPDSBB). Only those with late-onset disease (after 50 years of age) were included. The ethics committee from Dr. Manuel Gea González General Hospital authorized the study. Procedures were in accordance with the ethical standards of the Helsinki Declaration. A life-style interview was applied and written consent forms were signed prior to any intervention. Subjects were programmed for a fasting peripheral blood draw using the BD Vacutainer® collection system.

2.2. Genotyping methods

Samples were stored at 1°–6 °C. The DNA was extracted from peripheral blood leucocytes using the QIAamp DNA Blood Mini Kit® by QIAGEN® and quantified using a Thermo Scientific™ NanoDrop 2000 spectrophotometer. The H1/H2 haplotypes were determined analyzing the MAPT_238 bp deletion/insertion by PCR using the primer sequences GGAAGACGTTCTCACTGATCTG (forward) and AGGAGTCTGGCTTCA GTCTCTC (reverse). The 238 bp deletion was determined by the amplification of one distinct band at a size of 246 bp (H2/H2 haplotype); the amplification of two distinct bands (484 bp and 246 bp) corroborated the H1/H2 haplotype; and finally, the amplification of a 484 bp band only, corroborated the H1/H1 haplotype (Fig. 2).

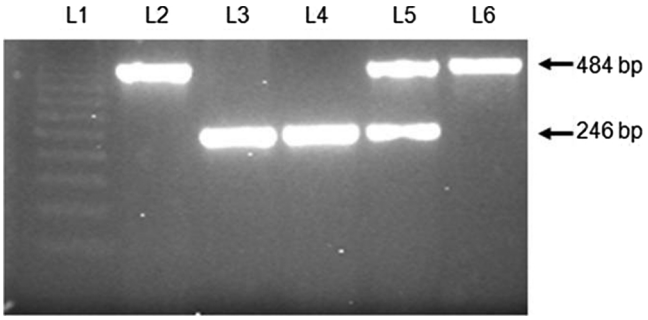

Fig. 2. Genotypification of H1/H2 haplotype using end point PCR (Genomic DNA in 3% agarose gel stained with ethidium bromide). L1, 50 bp DNA marker; L2 and L6, H1/H1 genotype; L3 and L4, H2/H2 genotype; L5, H1/H2 genotype.

2.3. Genotyping data analysis

For our genotypic analysis we utilized an on-line program provided by Institut Català d'Oncologia (https://snpstats.net/) as well as IBM SPSS Statistics for Windows (Version 21.0. Armonk, NY: IBM Corp.) for additional statistical analyses. For the purposes of this study p values < 0.05 were considered significant.

3. Results

3.1. General data about participants

In this work we included 108 cases and 108 controls. 39 paired cases resided in the central region versus 69 paired cases in the northern region. A total of 78 participants lived in the central region including Mexico City and surrounding states while 138 participants lived in the northern region including the city of Durango and rural areas of the state. The total age range of the participants was from 52 to 94 years with a mean age of 70.10 (± 9.16). Of these, 106 (49.1%) were women while 110 (50.9%) were men. For cases, the mean age at onset was 64.80 years (± 9.52). 18.51% of PD cases reported a family history of one or more first or second-degree relative(s) with PD. The mean evolution in years was 5.54 (± 4.11). Mean UPDRS III score was 43.00 (± 19.67), while total UPDRS score was 72.27 ± 33.29.

Table 1
Allelic and genotypic frequencies of H1/H2 haplotype and risk estimation to PD.

Haplotype	Controls n = 108	Cases n = 108	*p* value	OR (95% CI)	*p* value
H1	199 (0.92)	190 (0.88)	0.148*	1 (reference)	0.15
H2	17(0.08)	26 (0.12)		1.60 (0.84–3.04)	
H1/H1	92 (0.85)	84 (0.78)	0.363*	1 (reference)	0.36
H1/H2	15 (0.14)	22 (0.20)		1.60 (0.78–3.29)	
H2/H2	1 (0.01)	2 (0.02)		2.26 (0.20–25.78)	

* Pearson's Chi-squared is significant at $p \leq 0.05$.

3.2. Allelic and genotypic frequencies

The allelic and genotypic frequencies are shown (Table 1). Only one control and two cases presented the H2/H2 haplotype. No statistically significant differences were observed between groups in both, allelic (p = 0.148) and genotypic (p = 0.363) frequencies. The *odds ratio* estimation showed that neither the H2 allele (OR = 1.60, CI_{95} = 0.84–3.04) nor the H1/H2 (OR = 1.60, CI_{95} = 0.78–3.29) or H2/H2 (OR = 2.26, CI_{95} = 0.20–25.78) genotype are a risk factor for PD (Table 1).

3.3. Stratified allelic and genotypic frequencies

Subsequently, we stratified based on central or northern region from Mexico (Table 2). When comparing allelic frequencies in both controls and cases between regions we found no statistically significant differences (p = 0.098 and p = 0.595, respectively). Like the results observed when analyzing the total population, we found no differences in either the central or the northern region when comparing both allelic or genotypic frequencies between cases and controls (Table 2).

3.4. Stratified familial and sporadic PD allelic and genotypic frequencies

Lastly, we analyzed genotypic frequencies based on stratification by familial PD (n = 26) and sporadic PD (n = 82). No association for both familial PD (p = 0.48) or sporadic PD (p = 0.32) was observed (Table 3).

4. Discussion

4.1. Summary of previous studies

Although several studies have been carried out to evaluate the possible association between H1/H2 *MAPT* haplotype and PD, these have mainly been conducted in European populations. In this regard, in spite of the H1 haplotype being recognized as a risk factor for PD in caucasians [14], this association was not observed in German, Greek and Finnish populations [17,19], and thus suggests that it is ethnically dependent. With respect to Latin American populations, the only previous work was performed in population from the central region of Mexico, highlighting the need for more studies to determine the role of the H1/H2 haplotype in PD for these populations.

4.2. Regional genetic diversity in Mexican mestizo population and MAPT H1/H2

Mexican population, which is predominantly mestizo (composed of Amerindian, European, and, to a minor degree, African ancestries) has demonstrated regional genetic diversity that may affect biomedical traits in diseases [21,22]. Accordingly, our work included population from both the central and northern regions of the country. Although these genetic differences were reflected through a greater presence of H2 allele and H1/H2 genotype in cases and controls from the northern region compared to the central region, they were not statistically significant.

4.3. Multifactorial mechanisms for MAPT activation and PD?

We found no association between H1/H2 *MAPT* haplotype and PD risk, even after analyzing the population of each region independently. These results are consistent with those previously reported in Mexican mestizo population from the central region of Mexico [23] As PD is a multifactorial disease, perhaps our finding represents a distinct mechanism in the activation of *MAPT* in Mexican PD population; one that may very well be controlled by both genetic or epigenetic factors, including diet and environmental conditions. Future studies should consider additional analyses of current as well as new polymorphisms. Also, analysis of epigenetic changes of *MAPT* should be performed.

4.4. Study limitations

Finally, we would like to point out that our study has some limitations. We did not include a population from southern Mexico, which would allow a representation of the entire mestizo population of the country. Additionally, we did not determine the reported H1 subhaplotypes.

5. Conclusion

In conclusion, our results confirm no association between H1/H2 *MAPT* haplotype and PD in Mexican mestizo population and could serve as a useful reference when comparing among other ethnic groups in future studies.

Table 2
Allelic and genotypic frequencies of H1/H2 haplotype stratified by region and risk estimation to PD.

Region	Haplotype	Controls	Cases	*p* value	OR (95% CI)	*p* value
Central	H1	75 (0.96)	70 (0.90)	0.117*	1 (reference)	0.132
	H2	3 (0.04)	8 (0.10)		2.85 (0.72–11.20)	
	H1/H1	36 (0.92)	32 (0.82)	ND	1 (reference)	0.27
	H1/H2	3 (0.08)	6 (0.15)		2.25 (0.52–9.74)	
	H2/H2	0 (0)	1 (0.03)		ND	
Northern	H1	124 (0.90)	120 (0.87)	0.452*	1 (reference)	0.453
	H2	14 (0.10)	18 (0.13)		1.32 (0.63–2.79)	
	H1/H1	56 (0.81)	52 (0.75)	0.697*	1 (reference)	0.70
	H1/H2	12 (0.17)	16 (0.23)		1.44 (0.62–3.32)	
	H2/H2	1 (0.02)	1 (0.02)		2.26 (0.07–17.66)	

ND, not determined.
* Pearson's Chi-squared is significant at $p \leq 0.05$.

Table 3
Allelic and genotypic frequencies of H1/H2 haplotype stratified by familial and sporadic PD and risk estimation.

PD	Haplotype	Controls	Cases	*p* value	OR (95% CI)	*p* value
Familial	H1	47 (0.90)	45 (0.87)	0.539*	1 (reference)	0.541
	H2	5 (0.10)	7 (0.13)		0.68 (0.20–2.31)	
	H1/H1	21 (0.81)	20 (0.77)	ND	1 (reference)	0.48
	H1/H2	5 (0.19)	5 (0.19)		0.95 (0.21–4.36)	
	H2/H2	0 (.00)	1 (0.04)		ND	
Sporadic	H1	152 (0.93)	145 (0.88)	0.186*	1 (reference)	0.190
	H2	12 (0.07)	19 (0.12)		0.60 (0.28–1.28)	
	H1/H1	71 (0.87)	64 (0.78)	0.336*	1 (reference)	0.32
	H1/H2	10 (0.12)	17 (0.21)		0.52 (0.22–1.22)	
	H2/H2	1 (0.01)	1 (0.01)		0.88 (0.05–14.55)	

ND, not determined.
* Pearson's Chi-squared is significant at $p \leq 0.05$.

Contributions

EGM-M and JMS-P were involved in the experimental design and drafted the manuscript. OA-C and AS-C were involved in the experimental process and revised it critically for important intellectual content. EMM-H, FXC-J, and OL-L gave approval for the version to be published and were involved in revising it critically. LAR-C and GQ-C were involved in the clinical aspects of the study and revised the intellectual content of the manuscript.

Disclosure statement

The authors disclose no actual or potential conflicts of interest.

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Oxidative stress equilibrium during obstetric event in normal pregnancy

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Abstract

Objective: The aim of this study was to determine malondialdehyde (MDA) concentration as an oxidative stress marker and total antioxidant capacity (TAC) in pregnancy before and after perinatal event.
Methods: This study was performed on 200 healthy full-term pregnant women admitted to pregnancy resolution in Maternal-Child Hospital of Durango, Mexico. Oxidative stress and total antioxidant capacity were assessed through detection of lipid peroxidation by quantitation of thiobarbituric acid-reactive substances (TBARS) and total antioxidant capacity (TAC) through ferric reducing ability of the plasma (FRAP)
Results: Our results showed increased levels of MDA after vaginal delivery. Total antioxidant capacity was also increased after obstetric event, but it did not differ between vaginal delivery and caesarean section.
Conclusions: We demonstrated that MDA concentrations are increased two hours after obstetric event, and this increase correlates with VD. The TAC was increased as a compensatory mechanism during obstetric event. Another important finding is that women receiving analgesia administration in vaginal delivery, as well as dexamethasone administration in caesarean section, experienced a protector effect that decreased MDA levels.

Keywords: Delivery event, Oxidative stress, Malondialdehyde and Total antioxidant capacity.

Introduction

Oxidative stress represents a situation where there is an imbalance between the reactive oxygen species (ROS) and the availability and activity of antioxidants [1]. Due to the high potential to injure vital biological systems, ROS have been associated with more than one hundred diseases in their reproductive process. Several studies reported that parturition induces a strong oxidative stress response in mothers, implying an increased production of free radicals that must be controlled by their antioxidant system. This defence system can be overloaded during delivery in cases of abnormal oxygenation, where increased lipid peroxidation occurred [2]. In normal pregnancies, changes in oxygen concentrations are a very well-controlled phenomenon that has to provide a delicate balance between the metabolic needs of the placenta, foetus and mother, and the potential danger of ROS [3].

During the progression of normal labour, mothers experience a number of very stressful processes, such as pain, fear, anxiety and powerful contractions of the myometrium. The associated increase in intrauterine pressure and the degree of interruption or reduction in uteroplacental blood flow depend on the uterine contraction intensity [4], causing cycles of cellular hypoxia and reoxygenation; those are two essential elements of ischaemia–reperfusion injury [5].

The term oxidative stress has been defined as the “imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to damage [6]”. Importantly, it has been recently understood that ROS are the major signals involved in cardiovascular homeostasis [7].

Oxidative stress occurs in many pathological conditions and is now widely considered a major trigger of imbalance between protein synthesis and degradation [8]. Oxidative stress represents an inequity in oxidant and antioxidant levels. If there is an overproduction of oxidants that overwhelms antioxidant defences, oxidative damage of cells, tissues and organs ensues [9]. It has long been known that ROS are present in skeletal muscle [10] and can be generated during exercise and acute hypoxia periods [11]. As ROS can damage cell proteins, DNA and lipids through oxidation, they have been considered harmful agents. ROS production due to heavy exercise [12], as well as delivery process, have been shown to determine muscle damage, documented by increased lipid peroxidation. On the contrary, ROS production during moderate exercise causes positive adaptation, which is increased in insulin sensitivity, mitochondrial biogenesis and antioxidant defence systems [13]. When the production of ROS exceeds the capacity of antioxidant defence, oxidative stress has a harmful effect on the integrity of biological tissue through lipid peroxidation cascades or direct oxidation of membrane proteins. Malondialdehyde (MDA) is one of the small molecular weight pieces resulting from the fragmentation of polyunsaturated fatty acids undergoing attack by ROS and is generally accepted as an index of lipid peroxidation [14].

Material and Methods

Study population. This study was performed on 200 healthy full-term pregnant women admitted to pregnancy resolution in Maternal-Child Hospital of Durango, Mexico. The participants denied any chronic diseases, such as thyroid disease, liver disease, diabetes mellitus, hypertension or any pathological event during their pregnancy, including recent infections, premature membrane rupture and abnormal placentation. Written informed consent was obtained from all participants. The institutional Ethics Committee of Maternal-child Hospital approved the study. We obtained blood samples from the brachial vein (10 mL of blood) of each patient. The serum or plasma, obtained from centrifugation (3500 rpm for 10 min), was separated and distributed immediately in respective amounts necessary for marker determinations and stored at a temperature of -80°C, before carrying out the appropriate analysis.

Oxidative stress and total antioxidant capacity were assessed through detection of lipid peroxidation by quantitation of thiobarbituric acid-reactive substances (TBARS) and total antioxidant capacity (TAC) through ferric reducing ability of the plasma (FRAP).

TBARS assay. TBARS is a measurement of the reaction between MDA, a product of lipid peroxidation, and thiobarbituric acid (TBA) at temperatures of 90–100°C. The samples were assayed neat and in triplicate according to the method of Song et al. [15]. Briefly, 200 µL serum 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL of 20% acetic acid, 1.5 mL of 0.9% TBA and 0.6 mL of distilled water were added and vortexed. The reaction mixture was placed in a water bath at 95°C for 1 h. After cooling on ice, 1.0 mL of distilled water and 5.0 mL of butanol/pyridine mixture (15:1, v/v), were added and vortexed. After centrifugation at 10,000 rpm for 10 min, the pink MDA-TBA complex formed, and the resulting upper phase was determined at 532 nm. The concentration of TBARS was calculated using 1,5,3-tetraethoxypropane as a standard [15]. Results are expressed in nmol/L of MDA equivalents.

Total antioxidant capacity. The total antioxidant capacity was determined with the FRAP assay method, a simple test that measures the ferric reducing ability of plasma using 2,4,6 tripyridyl-s-triazine. Ferric to ferrous ion reduction at low pH causes a coloured ferrous-tripyridyltriazine complex to form. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions at a known concentration.

Statistical analysis

All tests were performed using IBM SPSS Version 21. Data were tested for normality distribution using the Kolmgorov–Smirnov test and appropriate distributional plots. Summary data are presented as mean ± standard error (SE) or median as appropriate for the distribution. Comparison concentrations of MDA and TAC between groups were made using the Student's t-test for independent groups. A p-value ≤0.05 was considered statistically significant.
The correlation tests were performed with Pearson's correlation and presented as p-value and ≤0.05

Results

Table 1 shows the socio-demographic and clinical characteristics of our study group. The mean age of women was 23.5 years, with a range of 14–43 years. The most frequent age range was 15–20 years (n=77, 38.5%). Thirty-three percent were primiparous, and 37% had three or more pregnancies. Socio-demographic differences between caesarean extraction (CE) and vaginal delivery (VD) groups were non-significant (Table 1).

Only 7% of women with VD requested epidural analgesia. In the case of CE, 83% received subarachnoid block, and 17% received epidural block. With respect to complications during delivery or surgical event, 95.5% of women did not present any complications, but 2% experienced obstetrical haemorrhage, 1.5% presented some type of dystocia event (shoulders or soft tissues) during delivery and 1% of these needed forceps.

All women received 10 UI of oxytocin in bolus immediately after VD or CE; women with CE (n=35) also received 20 mg of butilhioscina, 10 mg of metoclopramide, 60 mg of ketorolac and 8 mg of dexamethasone as surgical co-adjuvants. Women with obstetrical haemorrhage (n=4) received 800 µg of misoprostol, 0.2 mg of ergonovine maleate and 100 µg of carbetocin.

Hundred percent of newborns were alive, and 53.5% were female. The mean weight was 3.142 kg; 9.5% presented low birth weight, and 5% had macrosomy with respect to gestational age

Figure 1 showed that MDA concentrations were elevated after the obstetric event (2.43 ± 0.18 vs. 6.30 ± 0.37 nmol/L, p = 0.001). The comparison between VD and CE showed that MDA concentrations were significantly higher in VD group (6.6 ±5.5 vs 4.7 ±3.2, nmol/L p=0.001).

Plasma TAC concentrations were also elevated after the obstetric event (805.5 ±32.0 vs. 938.5 ±34.6 µmol/L, and a p-value=0.005) (Figure 2). In this case, the comparison between VD and CE showed that plasma TAC levels were slightly higher in the CE group (930.4±51.3 vs. 964±23.3 µmol/L) but without a statistic difference (p=0.616).

Discussion

The understanding of the role of free radicals in life sciences has consistently increased with time, and they have been connected to several physiological and pathological processes. ROS are generated from different exogenous and endogenous sources. The ROS produced in the tissues can inflict direct damage to macromolecules, such as lipids, nucleic acids and proteins. To counteract the harmful effects taking place in the cell, protection mechanisms have evolved to prevent and alleviate oxidative damages.

In pregnancy, many physiological changes occur in overall maternal systems, and the epidemiological characteristics can vary according to the geographical area. In our study, the women showed an average age of 23.5 years, similar to those reported nationally (24.2). However, our percentage of women aged between 14 and 20 (39.5%) years was higher than reported nationally (28.5%) [16]. These results show that our locality has a higher number of young pregnant women, a factor that has been associated with a more than doubled probability of maternal mortality by complications during pregnancy, intrapartum or postpartum period, in Mexico [17]. The delivery form in our study was mainly vaginal (83%), higher than reported by Heredia-Pi, L. et al. (53.1%) in Mexico [18]. With respect to BMI, we found that 34.5% of our study group was overweight or obese, similar to the results reported by Zonana-Nachach, A. et al. (38%) [19].

The increase in maternal lipid peroxidation and decrease of TAC is commonly associated with pathological conditions, such as preeclampsia or eclampsia [20]. On the other hand, the ROS increased slightly during normal pregnancy, suggesting it as a natural mechanism of uncomplicated pregnancies [21]. However, it is complicated to determine if this phenomenon is a cause or consequence of pregnancy.

In this study, the oxidative stress and total antioxidant capacity were assessed through detection of lipid peroxidation by quantitation of TBARS and TAC through FRAP two hours before and after perinatal event. We found a statistically significant difference in MDA levels ($p=0.001$) and plasma TAC concentrations ($p=0.001$) before versus after the perinatal event. Castro-Diaz, L. et al. [2] reported similar findings regarding oxidative stress status in pregnant women. They found an increase in plasma peroxides and erythrocyte membrane hydroperoxides, but a decrease in total antioxidant status during parturition, at three moments (at the beginning of the active phase of labour, at the start of expulsion and immediately after clamping cord). Induction of oxidative stress during periods of physical exhaustion, such as that experienced at the delivery moment, has been proposed as a cause of cellular membrane damage. This leads to an exacerbated inflammatory response and, therefore, pain and muscular fatigue [22]. Similar physiological processes that are present in women during pregnancy and postpartum independently of physical activities may be due to greater demand of oxygen [23]. In agreement with our results, we found an association between increased MDA levels in those women who had a VD measured two hours after obstetric event; these results differ from those reported by Hracsko, Z. et al. [24], who reported a higher concentration of MDA in women who had a pregnancy resolution by elective CE versus VD. However, it is important to note that Hracsko, Z. et al recollected blood samples at the time of delivery from umbilical cord. Our findings may be important because Arzalani-Zadeh, R. et al. [25] reported that MDA increased 24 h after surgical procedure. Similar results were reported by Zhang, G. L. et al. [26], who published that women under open surgical uterine myoma procedure had higher MDA values in serum versus the laparoscopic version of the same procedure, and that the levels of MDA in the women with open surgical procedure augmented at 24 h postsurgical procedure. So, we can say that MDA levels increase with time, and this fact may explicate our association with VD.

On the other hand, the negative association found between high MDA levels and dexamethasone administration in women whose pregnancy resolutions were by CE may be due to the anti-inflammatory effect of dexamethasone. This effect was demonstrated by Li, B. et al. [27] with an experiment in which rats with autoimmune encephalitis were administered dexamethasone, and the MDA levels decreased compared with a control group without disease and without dexamethasone. Another important association reported in this study is

the decrease of MDA levels in women who were administered analgesia during labour. This finding was also reported by Gyurkovits, Z. et al. [28], who compared the oxidative marker level in VDs with or without epidural analgesia. However, the impact of anaesthetics on oxidative stress is still not clear due to variations of patient's health conditions, types of surgery and quantities of anaesthetics.

Our findings showed increased TAC values after the obstetric event, independently of whether it was a VD or CE. However, Hracsko, Z. et al. also evaluated TAC with the same FRAP assay in pregnant women after delivery by VD or CE and found no differences between delivery forms, leading them to conclude that elective CE does not have an advantage over VD. The difference between our findings may be the moment the blood sample is collected. Additionally, the pregnant women were healthy, which may help to arrest the oxidative stress effect. The decrease in TAC status in women with pathological pregnancy, such as preeclampsia and gestational diabetes, is in agreement with results reported by Clerici, G. et al. [29]. Additionally, the statistically significant difference may be attributed to the effect of oxytocin administration in all women immediately after delivery and CE, as was reported by Akman, T. et al [30], who reported that administration of oxytocin significantly increased antioxidant capacity on rats.

Conclusion

Oxidative stress during the perinatal event can arise from overproduction of ROS by metabolic reactions that use oxygen and shift the balance between oxidant/antioxidant statuses in favour of the oxidants. However, diverse enzymatic mechanisms are also immediately activated to achieve an effective antioxidant response and offset ROS production to stop cellular damage and preserve maternal health. We demonstrated that MDA concentrations are increased two hours after obstetric event, and this increase correlates with VD. Another important finding is that pregnant women with analgesic administration during VD, or with dexamethasone administration in CE, experienced a protector effect that decreases MDA levels. On the other hand, the TAC was increased as a compensatory mechanism antagonist of ROS production and a protector system in human reproduction.

The birth of a new life carries in the mother generations of ROS molecules. However, it is surprising how maternal physiology produces compensatory antioxidant mechanisms for maintaining health and preserving reproductive function.

Finally, we consider it important to continue studying the oxidative/antioxidant status in pregnant women and their chosen methods of obstetric resolution. Greater understanding is necessary to determine the risk of development of chronic cardiovascular diseases and pathologies related to oxidative/antioxidant equilibrium.

Declaration of interest

The authors report no declarations of interest. This work was supported by Servicios de Salud de Durango and Universidad Juárez del Estado de Durango.

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Figure 1. MDA concentration 2 h before and after obstetric event.

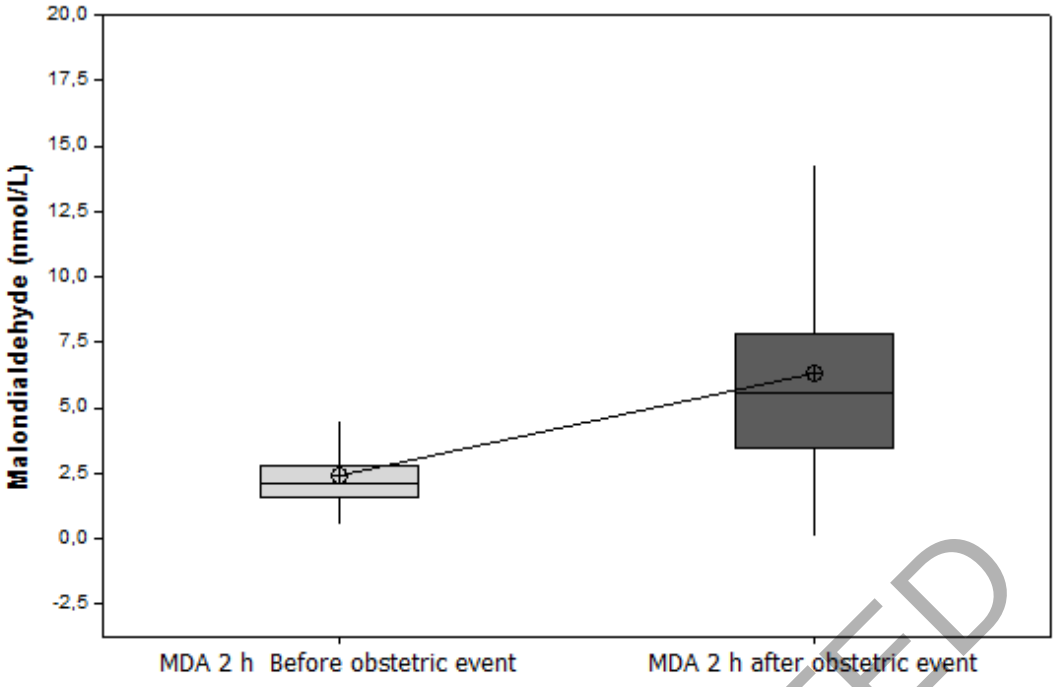

Figure 2. Plasma TAC concentration 2 h before and after obstetric event.

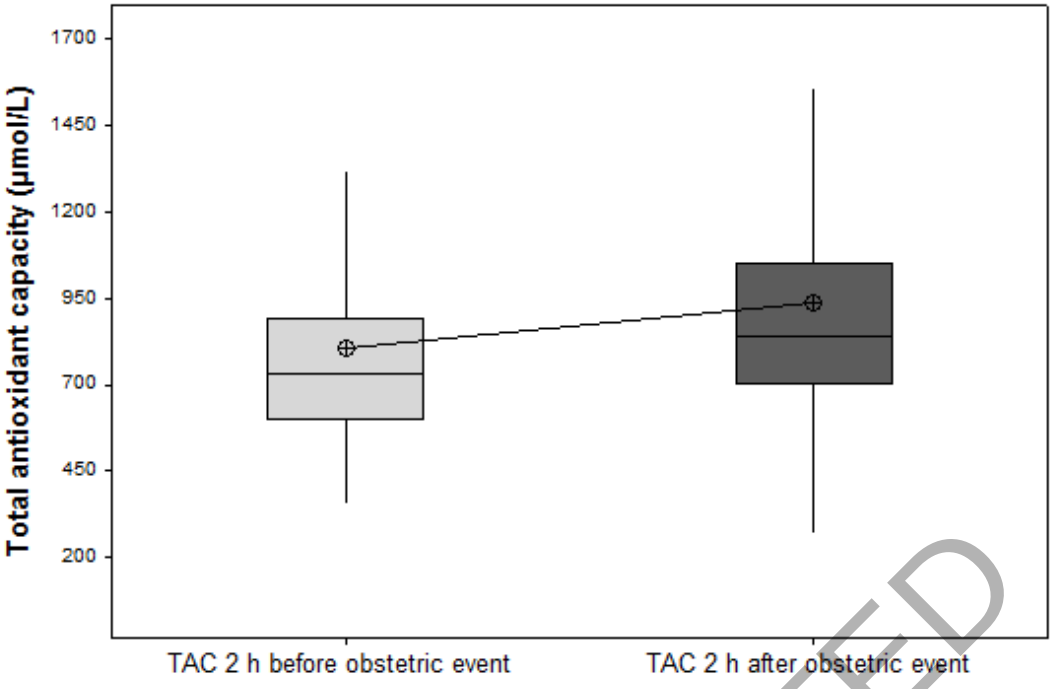

Table 1. Clinical and socio-demographic characteristics in pregnant women

Characteristic	Total (n=200)	Vaginal delivery (n=165)	Caesarean extraction (n=35)	p-value *
Weight (Kg)	60.2±12.3	59.2±11.8	65±13.7	0.327
Body mass index	23.5±4.5	23.2±4.4	25.1±4.8	0.688
Age (years)	23.6±6.1	23.5±6.3	24.1±5.4	0.480
Gestation age (weeks)	39.2±1.1	39.1±1.1	39.3±1.1	0.991
Newborn weight (kg)	3.14±0.4	3.1±0.41	3.29±0.35	0.181
Pregnancies number	2.34±1.3	2.39±1.4	2.09±1.0	0.072

* Student t-test
p≤0.05 is statistically significant

Prevalence of *Chlamydia trachomatis* Infection Diagnosed by Polymerase Chain Reaction in Female Sex Workers in a Northern Mexican City

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Purpose: We aimed to determine the association between *Chlamydia trachomatis* infection and female sex work, and the association between sociodemographic, obstetric, and behavioral characteristics of female sex workers and *C. trachomatis* infection.

Methods: Through a case–control study design, we studied 201 female sex workers and 201 age-matched women without sex work in Durango City, Mexico. *C. trachomatis* DNA was detected in cervical swab samples using polymerase chain reaction.

Results: *C. trachomatis* DNA was detected in 32 (15.9%) of the 201 cases and in 6 (3.0%) of the 201 controls (odds ratio [OR] = 6.15; 95% confidence interval [CI]: 2.5–15.0; $P < 0.001$). The frequency of infection with *C. trachomatis* in female sex workers did not vary ($P > 0.05$) regardless of the history of pregnancies, deliveries, cesarean sections, or miscarriages. Regression analysis of the behavioral characteristics showed that infection with *C. trachomatis* was associated only with consumption of alcohol (OR = 2.39; 95% CI: 1.0–5.71; $P = 0.04$).

Conclusions: We conclude that *C. trachomatis* infection is associated with female sex work in Durango City, Mexico. This is the first age-matched case–control study on the prevalence of *C. trachomatis* infection in female sex workers in Mexico using detection of *C. trachomatis* DNA in cervical samples.

Keywords: *Chlamydia trachomatis*, prevalence, female sex workers, case–control study

Introduction

Chlamydia trachomatis (*C. trachomatis*) is a ubiquitous, obligate, intracellular Gram-negative bacterial pathogen [1]. Humans are the only natural host of *C. trachomatis* [2]. This bacterium remains a significant public health burden worldwide [3]. It is estimated that 100 million cases of *C. trachomatis* infection occur annually [4]. *C. trachomatis* is the pathogen that is most often transmitted by sexual contact [5]. The clinical spectrum of infection with *C. trachomatis* varies from asymptomatic to several acute or chronic, local, and systemic diseases such as trachoma, oculo-genital, and neonatal diseases [6–7]. Infections with *C. trachomatis* of the lower female genital tract are frequently asymptomatic, undiagnosed, and untreated [4]. Pelvic inflammatory disease attributed to ascending genital *C. trachomatis* infections can result in ectopic pregnancies and infertility in women [4–5]. In addition, a recent meta-analysis demonstrated that individuals infected with *C. trachomatis* have a higher risk of cervical cancer [8]. *C. trachomatis* causes inclusion conjunctivitis of the newborn, with the female birth canal being the reservoir [9]. In addition, infection with *C. trachomatis* causes pneumonia and sepsis [10]. In men, *C. trachomatis* causes urethritis and epididymitis [11].

Little is known about the epidemiology of *C. trachomatis* infection in Mexico. The prevalence of *C. trachomatis* in women in Mexico has been determined in several population groups; for instance, 4% prevalence was reported in women in Cuernavaca City [12], 3.3% prevalence was found in women with leucorrhea in Mexico City [13], and 7.3% prevalence was reported in women in rural and suburban Oaxaca State [14]. However, female sex workers have shown higher prevalence of *C. trachomatis* infection. Uribe-Salas et al. [15] reported 11.1% prevalence of *C. trachomatis* in female sex workers in Mexico City. Whereas in 3 northern Mexican states, prevalence between 12.4% and 16.6% were found in female sex workers [16–17]. In the present study, by using a different study design (case–control) and laboratory method (polymerase chain reaction [PCR]) compared to those used in previous studies in female sex workers in Mexico, we aimed to determine the following: (1) the association between *C. trachomatis* infection and female sex work and (2) the sociodemographic, obstetric, and behavioral characteristics of female sex workers associated with *C. trachomatis* infection.

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Materials and Methods

Study Design and Women Studied. Through a case–control study design, we studied female sex workers (cases) and women with occupations other than sex work (controls) in Durango City from November 2014 to May 2016. Female sex workers were enrolled at the Sanitary Inspection Clinic of the Municipal Government in Durango City, Mexico. The inclusion criteria for enrollment of female sex workers were female sex workers (1) registered in the Sanitary Inspection Clinic, (2) worked in the sex industry for at least one year, and (3) aged ≥18 years old. Control subjects were women without sex work. Controls were sexually active women and enrolled at the Clinic for Family Planning in the Institute for Scientific Research. This public clinic is in Durango City, Mexico, and is part of Juárez University of Durango State.

Sociodemographic, Obstetric, and Behavioral Characteristics of Cases. We recorded the sociodemographic, obstetric, and behavioral data of cases with the aid of a questionnaire. Data about age, residence, birthplace, education, socioeconomic status, history of pregnancies, cesarean sections, miscarriages, and deliveries from all sex workers were obtained. Behavioral factors included duration (years) in the sex industry, area of work (urban, suburban, or rural), sex work in Mexican states other than Durango or abroad, contact with semen during vaginal intercourse, condom use, practice of oral or anal sex, injuries at sex work, and consumption of alcohol, tobacco or drugs.

DNA Extraction and Amplification. Cervical swab samples were obtained from participants and stored in a home-made 2SP (sucrose–phosphate) medium at −70 °C until analysis. DNA was extracted from cervical samples by proteinase K digestion and phenol–chloroform extraction. *C. trachomatis* DNA was detected by PCR using primers of the *omp* A gen of *C. trachomatis* [18]. These primers generate the amplification of a 129-base pair fragment. The amplification mixture contained 1.5 mM $MgCl_2$, 0.3 μM primers, 1 unit of Taq polymerase (Promega, Madison WI, EUA), 0.16 mM dNTPs, 2 ng DNA, and buffer in a 50-μL reaction volume. This amplification mixture was denatured at 95 °C for 10 min. Then, samples were amplified in 34 cycles. Each cycle consisted of the following: denaturation at 95 °C for 1 min, annealing at 61 °C for 2 min, and extension at 70 °C for 1 min. Samples underwent a final extension at 70 °C for 10 min. Amplification products were analyzed by electrophoresis in 2% agarose gel and stained with 0.1% ethidium bromide.

Statistical Analysis. Data were analyzed with the software SPSS version 15.0 (SPSS Inc. Chicago, Illinois). We calculated the odds ratio (OR) with 95% confidence interval (CI) to assess the association between *C. trachomatis* infection and sex work occupation. In addition, the association between *C. trachomatis* infection and the sociodemographic, obstetric, and behavioral characteristics of the female sex work were analyzed by the Pearson's chi-squared test and the Fisher exact test (when values were 5 or less). Variables of sex workers with a *P* value ≤0.20 obtained by bivariate analysis were further analyzed by multivariate analysis. We calculated the ORs and 95% CIs by regression analysis with the Enter method. Statistical significance was set at *P* value <0.05.

Ethics. The Ethics Committee of the Institute for Scientific Research of the Juarez University of Durango State, Mexico, approved this project. Participation in the study was voluntary. Written informed consent was obtained from all cases and controls before enrollment.

Results

Four hundred and two women were enrolled in this study. Half (n = 201) of them were cases (female sex workers), and 201 were controls (age-matched women). Mean ages in cases

Table 1. Sociodemographic characteristics of female sex workers and prevalence of *C. trachomatis* infection

	Women tested	Prevalence of *C. trachomatis* infection		
Characteristic	No.	No.	%	*P* value
Age groups (years)				
≤20	10	0	0.0	
21–30	82	14	17.1	0.56
31–40	64	11	17.2	
≥41	45	7	15.6	
Birth place				
Durango State	163	24	14.7	
Other Mexican state	38	8	21.1	0.33
Residence place				
Durango State	200	32	16.0	
Other Mexican state	1	0	0.0	1.00
Education (years)				
No education	4	0	0.0	
1 to 6	58	11	19.0	0.38
7–12	129	21	16.3	
>12	10	0	0.0	
Socio-economic status				
Low	39	8	20.5	
Medium	160	24	15.0	0.4

Table 2. Correlation between behavioral characteristics of female sex workers and *C. trachomatis* infection

	Women tested	Prevalence of *C. trachomatis* infection		
Characteristic	No.	No.	%	*P* value
Duration in the sex industry (years)				
1 to 5	20	1	5.0	0.14
6 to 11	47	11	23.4	
≥11	134	20	14.9	
Area of work				
Urban	196	32	16.3	0.74
Suburban	2	0	0.0	
Rural	1	0	0.0	
Sex activity in Mexican states other than Durango				
Yes	58	9	15.5	1.0
No	142	23	16.2	
Sex activity abroad				
Yes	3	1	33.3	0.4
No	198	31	15.7	
Condom use				
Yes	115	19	16.5	0.77
No	80	12	15.0	
Contact with semen during vaginal intercourse				
Yes	86	13	15.1	0.74
No	113	19	16.8	
Practice of oral sex				
Yes	121	16	13.2	0.18
No	79	16	20.3	
Practice of anal sex				
Yes	17	1	5.9	0.31
No	182	31	17.0	
Injuries during sex activity				
Yes	12	2	16.7	1.0
No	187	30	16.0	
Alcohol consumption				
Yes	36	10	27.8	0.03
No	165	22	13.3	
Tobacco consumption				
Yes	41	5	12.2	0.63
No	160	27	16.9	
Drug use				
Yes	13	3	23.1	0.43
No	188	29	15.4	

Table 3. Results of the regression analysis of selected behavioral characteristics of female sex workers and infection with *C. trachomatis*

Characteristic	Odds ratio	95% Confidence interval	*P* value
Duration in the sex industry (≥5 years)	0.31	0.03–2.43	0.26
Practice of oral sex	0.59	0.27–1.28	0.59
Alcohol consumption	2.39	1.0–5.71	0.04

and controls were 33.06 ± 9.76 (range: 18–67) years old and 33.08 ± 9.79 (range: 17–67) years old, respectively (P = 0.98). *C. trachomatis* DNA was detected in 32 (15.9%) of the 201 cases and in 6 (3.0%) of the 201 controls. The difference in the prevalence of *C. trachomatis* infection found in cases and controls was statistically significant (OR = 6.15; 95% CI: 2.5–15.0; $P < 0.001$). Bivariate analysis of socioeconomic characteristics of female sex workers showed no association ($P > 0.05$) between *C. trachomatis* infection and age, birthplace, residence, educational level, or socioeconomic status (Table 1). The frequency of infection with *C. trachomatis* in female sex workers did not vary ($P > 0.05$) regardless of the history of pregnancies, deliveries, cesarean sections, or miscarriages. With respect to behavioral characteristics, the variables, namely, duration in the sex industry, practice of oral sex, and alcohol consumption, showed P values ≤0.20 by bivariate analysis (Table 2), whereas other behavioral characteristics including area of work, sex work in Mexican states other than Durango or abroad, contact with semen during vaginal intercourse, condom use, practice of anal sex, injuries at sex work, consumption of tobacco, or drugs showed P values >0.20 by bivariate analysis. Regression analysis of the 3 behavioral characteristics with P values ≤0.20 obtained by bivariate analysis showed that the infection with *C. trachomatis* was associated only with consumption of alcohol (OR = 2.39; 95% CI: 1.0–5.71; P = 0.04) (Table 3).

Discussion

The epidemiology of *C. trachomatis* infection in female sex workers in Mexico has been scantly studied. We therefore aimed to determine the association between *C. trachomatis* infection and the occupation of female sex worker in Durango City, Mexico. We found a significantly higher frequency of *C. trachomatis* DNA in cervical samples from female sex workers than in those from control women without sex work occupation. This finding indicates that *C. trachomatis* infection is associated with the occupation of female sex worker. This age- and gender-matched case–control study thus demonstrates that female sex workers in Durango City have an increased risk of *C. trachomatis* infection. Comparison of our results with those reported in studies of female sex workers in Mexico is limited and should be interpreted with care because differences in the study design and in laboratory methods among the studies exist. There are few reports on the prevalence of *C. trachomatis* in female sex workers in Mexico. In a study performed in 3 cities (Durango, Zacatecas, and Torreón) in the north of Mexico, prevalence values between 5.2% and 16.2% of *C. trachomatis* infection were found [17]. However, in such study, a cross-sectional design and an enzyme immunoassay which detects *C. trachomatis* antigen in endocervix were used [17]. In the present study, we used a case–control study design, and diagnoses of *C. trachomatis* infection were based on *C. trachomatis* DNA detection using PCR. In a study of female commercial sex workers in Mexico City, researchers found a prevalence of 11.1% of *C. trachomatis* infection obtained by cultures of the bacterium in McCoy cells and identification with fluoresceinated monoclonal antibodies [15]. In an additional study, the same research group found 14.4% prevalence of *C. trachomatis* infection in female sex workers at the Mexico–Guatemalan border by analyzing cervical swab samples using a nonradioactive nucleic acid hybridization assay [19]. In a further study, 25.0% of female commercial sex workers in Mexico City had anti-*C. trachomatis* IgG antibodies [20]. However, our results obtained by detection of *C. trachomatis* DNA cannot be compared with those of seroprevalence of *C. trachomatis* infection. We compared our results with those obtained in studies of female sex workers in other countries using PCR methodology. The prevalence (15.9%) of *C. trachomatis* infection found in our study is higher than the 6.6% prevalence of *C. trachomatis* endocervical infection reported in female sex workers in Hungary using plasmid PCR [21]. In contrast, the prevalence found in our study is lower than a 28.5% prevalence of *C. trachomatis* cervical infection found in female sex workers in Dakar, Senegal, using PCR [22]. The association between *C. trachomatis* infection and female sex workers found in our study agrees with the same association found in a study in Bangladesh, where researchers found a significantly higher prevalence value of *C. trachomatis* infection in 40 female sex workers (58%) than in 110 sexually active women (27%) using immunochromatography test and/or plasmid PCR [23].

We found an association between *C. trachomatis* infection and alcohol consumption in female sex workers. In a search of this association in the biomedical literature, we did not find any report. It is possible that alcohol consumption in female sex workers might be linked to risky behavioral factors for *C. trachomatis* infection. Further research about this association should be conducted.

Conclusions

We conclude that *C. trachomatis* infection is associated with female sex work in Durango City, Mexico. This is the first age- and gender-matched case–control study on the prevalence of *C. trachomatis* infection in female sex workers in Mexico using detection of *C. trachomatis* DNA in cervical samples. The association between *C. trachomatis* and consumption of alcohol deserves further investigation.

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Authors' Contributions

LFSA and NVH conceived the study protocol, performed the data analysis, and wrote the manuscript. FMGI and JHT performed data analysis and wrote the manuscript. JANF obtained the samples and clinical data. MAD, ARPA, AASC, and EIAS performed laboratory tests and data analysis. SEM performed statistical analysis. CAE participated in the design of the protocol, performed data analysis and wrote the manuscript. All authors read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that no conflict of interest exists.

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RESEARCH ARTICLE Open Access

Arsenic exposure and risk of preeclampsia in a Mexican mestizo population

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Abstract

Background: Exposure to arsenic in drinking water has been associated with various complications of pregnancy including fetal loss, low birth weight, anemia, gestational diabetes and spontaneous abortion. However, to date, there are no studies evaluating its possible association with preeclampsia.

Methods: This case–control study involved 104 preeclamptic and 202 healthy pregnant women. The concentrations of arsenic in drinking water and urine were measured using a Microwave Plasma-Atomic Emission Spectrometer.

Results: We found relatively low levels of arsenic in household tap water (range of 2.48–76.02 μg/L) and in the urine of the participants (7.1 μg/L vs 6.78 μg/L in cases and controls, respectively).

Conclusions: The analysis between groups showed for the first time that at these lower levels of exposure there is no association with preeclampsia.

Keywords: Preeclampsia, Arsenic, Drinking water

Background

Preeclampsia (PE) is a disorder peculiar to pregnancy and a major cause of maternal death and adverse fetal outcome [1]. In developing countries where access to health care is limited, PE is a leading cause of maternal mortality, with estimates of more than 60,000 maternal deaths per year [2] Although the exact pathophysiologic mechanisms of PE remain elusive, studies to date have implicated multiple processes, including the following: abnormal trophoblastic invasion, vasospasm, platelet activation, imbalance in the vasomotor-regulating factors and placental ischemia [3]. PE is characterized by increased oxidative stress due to the imbalance between lipid peroxidation and antioxidant defense mechanisms, leading to endothelial dysfunction and free radical mediated cell injury [4].

Arsenic-contaminated drinking water represents a major public health problem internationally [5–8]. The World Health Organization (WHO) and U.S. Environmental Protection Agency (EPA) standard for arsenic level in drinking water is 10 μg/L [9, 10]. Arsenic (As) is an established carcinogen and is also associated with a wide range of other chronic illnesses, such as diabetes, hypertension, and vascular diseases [11].

Oxidative stress has been identified as an important mechanism of As toxicity and carcinogenicity. In particular, As induces oxidative DNA damage and lipid peroxidation [12–16]. Oxidative stress and disrupted antioxidant systems have been shown to be involved in a wide range of pregnancy complications such as impaired fetal growth, PE, and miscarriage [17, 18].

Besides the generation of oxidative stress as a possible mechanism by which As may be associated with PE, Shin Le et al. reported that exposure to environmentally relevant concentrations of As (2.5 μM of AsNaO2) inhibit the migration of EVT cells (a human extravillous trophoblast cell line) in vitro, therefore, a similar mechanism may be occurring in vivo [19].

Several studies have been conducted to determine the association between chronic As exposure and adverse pregnancy outcome. Excess spontaneous abortion,

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stillbirth, and preterm birth rates among women with chronic As exposure have been reported [20–23]. However, to date there are no reports that show an association between As exposure and PE. This study evaluates whether As exposure from drinking water is associated with PE in a population of northern Mexico.

Methods

Patient recruitment

This prospective case–control study was approved by the Research Ethics Committee of the General Hospital of the Ministry of Health of Durango, Mexico in accordance with the Code of Ethics of the Declaration of Helsinki. Signed informed consent was obtained from all patients and controls before participation in the study. The sample size was calculated using the formula $n = (Z_{\alpha/2} + Z_{\beta})^2\ \dot{p}\ (1-\dot{p})\ (r+1)/d^2r$. The n needed to achieve 80 % power with an alpha of 0.05 was 94 (cases) and 188 (controls). Finally, we recruited 104 women diagnosed with PE (cases) and 202 healthy pregnant women (controls). The inclusion criteria were all those women diagnosed with mild PE (blood pressure (BP) ≥ 140/90 mmHg and proteinuria ≥ 30 mg/dL), severe PE (BP ≥ 160/110 mmHg and proteinuria ≥ 2000 mg/dL) and eclampsia (defined as occurrence, in a woman with PE, of seizures that cannot be attributed to other causes). The control group was conformed by healthy pregnant women attending the same hospital; without hypertensive, pathological or metabolic disorders during pregnancy. Follow up was given to the control group to corroborate the normality of the blood pressure values.

Sample collection

Within 1–3 weeks of delivery, a drinking water sample was collected at the homes of each of the study participants. Drinking-water samples were collected based on the subject's primary drinking water source. Maternal spot urine samples were collected at the hospital before delivery and immediately transported to the laboratory. Samples were stored at −80 °C until processing.

Detection of As in drinking water and urine

The concentrations of As in drinking water (DW) and urine were measured in the toxicology laboratory of Scientific Research Institute of the Universidad Juárez del Estado de Durango (UJED) using a Microwave Plasma-Atomic Emission Spectrometer (MP-AES 4100). The Trace Elements in Water standard reference material (SRM 1643e) (National Institute of Standards and Technology, Gaithersburg, MD) was used for quality control. The limit of detection for As in DW by MP-AES was 0.5 μg As/L. For urine analysis, six point calibration curves were prepared. To compensate for variation in the dilution of the urine (caused by variation in fluid intake, time of sampling, temperature, and physical activity), we adjusted the concentrations by specific gravity.

Statistical analysis

Independent sample Student's *t*-tests were performed using SPSS software (version 15.0; SPSS Inc., Chicago, IL, USA). Odds ratios (ORs) as estimates of relative risk of the disease were calculated with 95 % confidence intervals (95 % CIs). The ORs were adjusted for variations in age and weeks of pregnancy by means of a multivariate logistic regression model. Mann–Whitney *U* test was used when the data were not normally distributed. For analysis, our patients were stratified into 3 groups based on As levels in DW (Table 3). The Group 1 (G1) presented levels lower than 10 μg/L, group 2 (G2) levels between 10.1 μg/L and 25 μg/L and group 3 (G3) levels above 25.1 μg/L

Results

Clinical characteristics for controls and cases are shown in Table 1. Of the 104 women diagnosed with PE, 13 had mild PE, 72 severe PE and 19 eclampsia. Variables that showed a difference between groups were family history of PE, systolic and diastolic blood pressure (mm Hg), weeks of pregnancy and body mass index (Table 1). The range of As concentration in household tap water was 2.48–76.02 μg/L with more than 95 % of the participants having As levels higher than 10 μg/L. The mean concentration of As in DW was 39.58 μg/L and 40.49 μg/L for cases and controls, respectively; there were no statistically significant differences (Table 2, $p = 0.816$). While the WHO sets a maximum concentration of 10 μg/L in DW, the authorities in Mexico have set a maximum concentration of 25 μg/L (NOM-127-SSA1-1994) [24]. For this reason, the OR was estimated stratifying our patients into 3 groups based on As levels in DW. The results of Table 3 show that although the group exposed to concentrations above 25 μg/L presents an increased risk (OR = 1,715). This difference is not statistically significant ($p = 0.214$).

Table 1 Clinical characteristics for cases and controls

Clinical features	Controls (*n = 202*)	Cases (*n = 104*)	P-*value*
Age (years)	24.30 (7.078)[a]	24.39 (7.349)[a]	.92[b]
Weeks of pregnancy	37.49 (3.96)[a]	35.82 (3.97)[a]	0.001[b]
Systolic BP (mm Hg)	111.74 (10.82)[a]	158.36 (16.41)[a]	<0.0001[b]
Diastolic BP (mm Hg)	70.39 (9.97)[a]	101.21 (10.3)[a]	<0.0001[b]
Number of pregnancies	2.26 (1.40)[a]	2.34 (2.49)[a]	0.718[b]
Body mass index	24.61 (5.22)[a]	27.63 (5.82)[a]	<0.0001[b]
PE antecedent	13/202	14/104	0.045[c]

[a]Mean ± Standard deviation
[b]Independent sample *T* test
[c]Chi square test

Table 2 Water and urine arsenic levels in cases and controls

Arsenic μg/L	Controls (*n = 202*)	Cases (*n = 104*)		P-*value*
Water	40.49 (16.40)[a]	39.58 (26.43)[a]		0.816[b]
Urine	6.78 (3.48)[a]	7.1 (5.74)[a]		0.428[c]
		Mild PE *n* = 13	Severe PE/eclampsia *n* = 91	P-*value*
Water		46.03 (20.65)[a]	38.62 (26.87)[a]	0.519[b]
Urine		7.82 (6.87)[a]	7.03 (5.67)[a]	0.788[c]

[a] Mean ± Standard deviation
[b] Independent sample *T* test
[c] Mann–Whitney *U* test

Total urinary As concentration (U-tAs) was also evaluated. The mean concentration of U-tAs was 7.1 μg/L and 6.78 μg/L for cases and controls, respectively; there were no statistically significant differences (Table 2, $p = 0.428$). With the intention to establish whether As may be associated with the severity of PE, the cases were stratified in mild PE and severe PE/eclampsia. The results of Table 2 show that there is no statistically significant differences in the U-tAs (p =0.788). The risk of PE by U-tAs was estimated piling up to the patients in tertiles. The results in Table 3 show that at these levels, U-tAs is not a risk for PE.

Finally, we evaluated the correlation between As in DW and U-tAs. We observed an increase in the U-tAs associated with higher levels of As in DW. G1 presented a mean of 3.39 μg/L, G2 of 6.67 μg/L and G3 of 7.8 μg/L. However, the correlation coefficient was very low ($R^2 = 0.036$).

Discussion

To our knowledge this is the first study that evaluates if As exposure from DW is associated with PE. The As concentrations in household tap water (2.48–76.02 μg/L) were consistent with those previously found by our working group in the wells that provide DW to the city of Durango [25, 26]. Although these concentrations are not as high as those reported in other countries [27–30] or even in other regions of our own locality [31], there is a tremendous interest in the evaluation of regions with low or moderate As exposure in accordance with the increasingly clear evidence that relatively low levels of As can have health effects. Our comparative analysis between controls and cases evidenced no statistically significant differences. In addition, no differences were found in the analysis based on the severity of the PE.

The analysis of U-tAs showed a mean of 7.1 μg/L for cases and 6.78 μg/L for controls. These U-tAs levels are clearly lower than those reported among pregnant women in Bangladesh (80 μg/L) [32] and even lower than those reported in pregnant women in the nearby region known as Comarca Lagunera (23.3 μg/L) [33]. In our study we didn't find an association between U-tAs and PE or an association with the severity of PE. Recently, Joy-Mendez et al. found no association between serum As levels and blood pressure in a cohort of pregnant women from Mexico city [34]. They reported a mean of 15.2 μg/L of As in serum. Although they don't evaluate PE, our results can be considered similar.

In contrast to our results, several reports have associated As exposure with pregnancy complications including low weight of the newborn [35], fetal death [36], gestational diabetes [32], anemia [37] and spontaneous abortions [38], however, these associations appear at significantly higher levels of As (e. g., fetal death, U-tAs >200 μg/L or spontaneous abortions, As in DW >100 μg/L).

Our results could be interpreted on the one hand, as a confirmation of no association between As and PE, at least at these low levels. On the other hand, they might suggest that we need higher levels of As exposure to be able to observe the association.

Our study has some limitations. Although the participants state that their main source of water is from the tap, we can't rule out that As can come from other sources of drinking water (e.g., bottled water), some

Table 3 Odds ratio estimation by ranges of arsenic in water and urine

Water arsenic	OR* (95 % CI)	P-*value*	Urine arsenic	OR* (95 % CI)	P-*value*
Group 1[a] *n = 10*	Reference		Tertile 1[d] *n = 102*	Reference	
Group 2[b] *n = 69*	1.486 (0.200–11.025)	0.698	Tertile 2[e] *n = 102*	1.400 (0.748–2.621)	0.698
Group 3[c] *n = 227*	1.715 (0.732–4.019)	0.214	Tertile 3[f] *n = 102*	0.788 (0.411–1.512)	0.214

[a] DW As < 10 μg/L
[b] DW As 10.1–25 μg/L
[c] DW As >25 μg/L
[d] U-tAs ≤7.4956 μg/L
[e] U-tAs >7.4956 ≤ 11.4911 μg/L
[f] U-tAs >11.4911 μg/L
* ORs were adjusted for age and weeks of pregnancy

food, or by some occupational exposure. Another limitation is that we didn't find high levels of U-tAs, so we can't establish in our study if higher levels of urinary As are or are not associated with PE.

The evaluation of pregnant women with higher levels of As as well as the analysis of other factors (e.g., genetic or nutritional) becomes necessary to confirm and strengthen our findings.

Conclusions

First, it is shown that the majority of our population is exposed to As levels higher than that established by the WHO. In addition, our work suggests for the first time that there is no association between As exposure and PE.

Abbreviations

As, arsenic; DW, drinking water; EPA, environmental protection agency; ORs, odds ratios; PE, preeclampsia; UJED, Universidad Juárez del Estado de Durango; U-tAs, urinary arsenic concentration; WHO, World Health Organization

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Availability of data and materials

The data will not be shared in order to protect the participants' anonymity.

Authors' contributions

EMMH, ATV and OLL carried out the statistical analysis and helped to draft the manuscript. EIAS, SMSP, FVA and MBS carried out the integration of groups and sampling of household tap water. FXCJ and MAD carried out the arsenic determinations. JMSP and ASC conceived of the study, and participated in its design and coordination and drafted the manuscript. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of the General Hospital of the Ministry of Health of Durango, Mexico. Informed signed consent was obtained from study participants.

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RESEARCH ARTICLE

TNF-α Polymorphisms and Maternal Depression in a Mexican Mestizo Population

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Abstract: ***Background***: Depressive disorders are common during pregnancy. There is compelling evidence that the inflammatory response system is important in the pathophysiology of depression. Higher concentrations of proinflammatory cytokines including tumor necrosis factor-alpha (TNF-α) in depressed subjects have been described. Because several polymorphisms in the TNF-α promoter region are known to affect its gene expression, the aim of this study was determine whether TNF-α -857C/T, -308G/A, and -238G/A polymorphisms confer susceptibility to depression during pregnancy in a Mexican mestizo population.

Methods: This case-control study involved 153 depressed pregnant women and 177 controls. Polymorphisms were genotyped using real-time PCR. Odds ratios (OR) and 95% confidence intervals adjusted by age, body mass index, number of pregnancies, months of pregnancy and number of abortions were used to estimate risk.

Results: The -857CT genotype was found to increase the risk for depression (OR= 1.73, 95% CI= 1.06-2.82). In contrast, the -238GA genotype reduced the risk (OR= 0.33, 95% CI= 0.14-0.72). The -308G/A polymorphism was not associated with risk for depression. Finally, the C857-G308-A238 haplotype was associated with a decreased risk of depression (OR= 0.35, 95% CI= 0.15-0.82).

Conclusion: Our results show for the first time an association between *TNF-α* -857C/T and -238G/A polymorphisms and prenatal depression in Mexican mestizo population.

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1. INTRODUCTION

Depression during pregnancy can lead to behavioral changes such as the abandonment of prenatal controls, poor adherence to medical indications, consumption of tobacco, drugs, and alcohol with potentially devastating consequences for both mother and baby. Almost 10% of pregnant women are diagnosed with depression. This percentage may be increased depending on cultural and socioeconomic conditions [1]. A number of factors have been associated with depression during pregnancy: lack of family or social support, stressful life events, tobacco use, hormonal changes, anxious temperament, history of mental illness and genetic predisposition, are some examples [2].

Besides this, several reports suggest that depression is an inflammatory disorder mediated by proinflammatory cytokines, such as interleukins 2, -6, and -12 and tumor necrosis-alpha (TNF-α) [3-5].

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TNF-α is a proinflammatory cytokine that strongly contributes to inflammatory and immune responses by inducing a cascade of various inflammatory cytokines; it is produced by monocytes, macrophages and T and B lymphocytes, and also by microglia in the central nervous system [6].

The role of TNF-α in depression has been evaluated in both epidemiological studies and animal models. A meta-analysis showed that TNF-α is commonly elevated in depressed patients [7]; also, the administration of TNF-α to rats induces a spectrum of behavioral changes including social withdrawal, decreased motor activity, reduced food intake and sleep alterations [8].

The TNF-α gene is located on chromosome 6p21.3, within the class III region of MHC [9, 10]. Single nucleotide polymorphisms (SNPs) in the TNF-α gene, including -238G/A (rs361525), -308G/A (rs1800629), and -857C/T (rs1799724) have been described [11]. Because these SNPs in the TNF-α promoter region have been associated with different TNF-α expression profiles and circulating TNF-α levels, they can modulate inflammatory processes, disease development and response to treatment [12]. For that reason, the main goal of our study determined if -238G/A, -308G/A and -857C/T polymorphisms of TNF-α gene confer susceptibility to depression during the prenatal period in a Mexican mestizo population.

2. MATERIALS AND METHOD

2.1. Patient Recruitment

Blood samples were obtained from patients of a previous study conducted at General Hospital of the Secretary of Health in Durango City from March 2015 to February 2016 [13].

2.2. DNA Extraction and Genotyping of Samples

The DNA extraction from blood samples was performed using the QIAamp DNA blood extraction kit (Qiagen, Hilden, Germany). The genotypes were assessed using TaqMan assays (Applied Biosystems) as described previously [14]. The predesigned assays were C-11918223-10, C-7514879-10, and C-2215707-10 (-857C/T, -308G/A, and -238G/A, respectively.

2.3. Statistical Analysis

The clinical characteristics were expressed as mean and were compared using the Student's *t*-test. The allele and genotype frequencies were calculated by direct counting. Deviation from the Hardy-Weinberg equilibrium (HWE) constant was tested using a χ^2 test with 1 degree of freedom. The differences of distributions of the polymorphisms were performed by χ^2 analysis using SPSS software (version 15.0; SPSS Inc., Chicago, IL, USA); $p < 0.05$ was considered statistically significant. The odds ratio was calculated from allelic and genotype frequencies with 95% confidence intervals (95% CI) using the SNPstats software program (Catalan Institute of Oncology).

3. RESULTS

A total of 330 pregnant women were enrolled in the study (153 depressed pregnant women and 177 controls). Of the 153 women diagnosed with depression, 93 had mild depression and 60 severe depression. Only the body mass index showed a difference between groups ($p = 0.036$, Table **1**).

Allelic and genotypic frequencies of -857C/T, -308G/A, and -238G/A TNF-α polymorphisms are shown in Table **2**. All polymorphisms were in HWE. The allelic frequencies of -857C/T and -238G/A showed statistically significant differences between groups ($p= 0.030$ and $p= 0.0019$, respectively). Also, these differences were observed in the genotypic frequencies of -857C/T polymorphism ($p = 0.047$). No differences in allelic or genotypic frequencies between cases and controls were observed in -308G/A polymorphism (Table **2**, $p > 0.05$).

The risk of depression by the presence of these polymorphisms was determined. A logistic regression model adjusted for age, body mass index, number of pregnancies, months of pregnancy and number of abortions was used. The results of Table **3** showed that the -857CT genotype is a risk factor (OR= 1.73, 95% CI= 1.06-2.82) and that the -238GA genotype is a protector factor (OR= 0.33, 95% CI= 0.14-0.72) for depression in pregnant women. Furthermore, a significant trend was observed for both polymorphisms (p for trend = 0.035 and 0.001). Finally, haplotype analysis showed that the C857-G308-A238 haplotype was significantly associated

Table 1. Clinical characteristics of depressed (cases) and healthy pregnant women (controls).

Clinical Features	Cases, *n* = 153	Controls, *n* = 177	*p*-value
Age (years) [a]	23.49 (8.72)	23.58 (8.05)	0.925 [b]
BMI [a]	27.99 (5.71)	26.71 (5.32)	0.036 [b]
Number of pregnancies [a]	2.21 (1.52)	2.12 (1.51)	0.606 [b]
Months of pregnancy [a]	6.54 (1.52)	6.82 (1.42)	0.086 [b]
Number of abortions [a]	0.13(0.38)	0.17 (0.49)	0.439 [b]

[a]Mean (± Standard deviation).

[b]Independent sample *T* test.

Table 2. Allele and genotype frequencies of TNF-α polymorphisms in depressed pregnant women (cases) and healthy pregnant women (controls).

		Cases *n* = 153	Controls *n* = 177	*p*-value
-857C/T	C	0.77	0.84	0.030 [a]
	T	0.23	0.16	
	C/C	0.59	0.72	0.047 [a]
	C/T	0.37	0.25	
	T/T	0.04	0.03	
-308G/A	G	0.94	0.94	0.929 [a]
	A	0.06	0.06	
	G/G	0.89	0.90	0.175 [a]
	G/A	0.11	0.08	
	A/A	0	0.02	
-238G/A	G	0.97	0.92	0.0019 [a]
	A	0.03	0.08	
	G/G	0.95	0.84	ND [a]
	G/A	0.05	0.16	
	A/A	0	0	

[a] Pearson's Chi-squared is significant at $p \leq 0.05$.

ND, not determined.

Table 3. TNF-α polymorphisms association with depression in pregnant women.

		Cases n	Controls n	OR	95% CI	p-value
-857C/T	C/C	90	126	1.00	(referent)	0.078
	C/T	57	45	1.73	(1.06-2.82)	
	T/T	6	6	1.54	0.44-5.40	
	p value for trend					0.035
-308G/A	G/G	136	160	1.00	(referent)	0.14
	G/A	17	14	1.42	0.66-3.05	
	A/A	0	3	ND	ND	
	p value for trend					0.960
-238G/A	G/G	145	148	1.00	(referent)	0.0035
	G/A	8	29	0.33	0.14-0.72	
	A/A	0	0	ND	ND	
	p value for trend					0.001

ND, not determined.

with a decreased risk of depression (OR= 0.35, 95% CI= 0.15-0.82, Table **4**).

4. DISCUSSION

The continued search for risk markers in depressed pregnant women remains of great interest because of the wide range of negative outcomes such as social isolation [15], marital discord [16], child delays in motor or intellectual development [17], restricted fetal growth and elevated stress reactivity in infants [18, 19], among others.

Peripheral inflammation can lead to depression through several immune-mediated pathways that transmit the signal from the periphery to the central nervous system. Patients with major depressive disorder exhibit all of the cardinal features of an inflammatory response, including increased

Table 4. Frequencies and association of TNF-α (−857C/T, −308G/A and -238G/A) haplotypes with depression in pregnant women.

Haplotypes	Cases	Controls	OR	95% CI	*p*-value
C857-G308-G238	0.69	0.70	1.00	(referent)	
T857-G308-G238	0.225	0.161	1.39	(0.91 - 2.12)	0.12
C857-A308-G238	0.054	0.056	1.03	(0.53 - 2.00)	0.93
C857-G308-A238	0.024	0.081	0.35	(0.15 - 0.82)	0.016
C857-A308-A238	0.001	0	ND	ND	ND

ND, not determined.

expression of pro-inflammatory cytokines and their receptors and increased levels of acute-phase reactants, chemokines and soluble adhesion molecules in peripheral blood and cerebrospinal fluid [20]. In this context, SNPs that modulate the expression of TNF-α or any other pro-inflammatory cytokine may have a potential role in susceptibility to depression.

The polymorphisms evaluated in this work have previously been associated with differences in TNF-α gene expression. Furthermore, they also have been associated with some disorders including attempt suicide [21], schizophrenia [22], obsessive-compulsive disorder [23], major depressive disorder [24] and post-stroke depression [25]. However, there are no studies evaluating their possible role in prenatal depression.

Our results showed that the -857CT and -238GA genotypes increase and reduce the risk to develop depression in our population, respectively. These results are consistent with evidence suggesting an increase in proinflammatory cytokines in depressed patients. The TNF-α-857T allele was reported to be associated with high TNF-α production in *in vitro* cell proliferation studies [26]. Also, the T allele was associated with increased transcription of TNF-α in a Chinese population and high serum levels of TNF-α in the Indian and Japanese population [12, 27-29]. On the other hand, the −238A allele was reported to down-regulate TNF-α expression [30, 31]. Moreover, the −238G allele was related to high TNF-α mRNA expression and high serum TNF-α concentrations in rheumatoid arthritis and in knee osteoarthritis patients [32, 33].

Concerning -308G/A SNP, the literature is controversial. Studies with both increased [34-36] and decreased [37-40] TNF-α plasma or mRNA levels associated with the −308A allele have been published. Also, some works suggest an association of this SNP with depression [24, 25] but others not [41, 42]. Our results suggest no association between the -308G/A SNP and prenatal depression.

In relation to the genotypic frequencies, previous studies reported a frequency of 0.746 CC, 0.248 CT and 0.004 TT for -857CT SNP, 0.93 GG and 0.07 GA for -308G/A SNP and 0.89 G/G and 0.11 G/A for -238G/A SNP [32, 43]. We found very similar results for our group of controls.

Interestingly, the C857-G308-A238 haplotype was associated with a decreased risk of depression. As already mentioned, we would expect higher levels of proinflammatory cytokines in patients with depression. Therefore, our results suggest that the presence of the C857 allele (associated with decreased transcription of TNF-α) would have a greater effect than that of the C857 allele (associated with increased transcription of TNF-α) and consequently, lower amounts of TNF-α protein would be produced in those individuals who have the C857-G308-A238 haplotype. However, further experimentation will be needed to prove it.

There are some limitations in our study. First, the effect of SNPs on TNF-α gene expression or circulating TNF-α levels was not evaluated. These data would be of great interest, in particular regarding the controversial -308G/A SNP findings in previous reports. Second, only pregnant women from the northern region of México were included. To establish these polymorphisms as risk markers in the general Mexican population, it will be necessary to carry out additional studies that include women from all regions of the country, based on the demonstrated genetic differences between subpopulations from different regions throughout México [44].

CONCLUSION

Our results show for the first time an association between TNF-α -857C/T and -238G/A polymorphisms and prenatal depression in a Mexican mestizo population.

LIST OF ABBREVIATIONS

HWE = Hardy-Weinberg Equilibrium

SNP = Single Nucleotide Polymorphism

TNF-α = Tumor Necrosis Factor-alpha

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Ethics Committee of the General Hospital of the Secretary of Health in Durango City, Mexico approved this study, and written informed consents were obtained from all participants and from the next of kin of minor participants.

HUMAN AND ANIMAL RIGHTS

The study was conducted in accordance with the Helsinki Declaration.

CONSENT FOR PUBLICATION

Written informed consents were obtained from all participants and from the next of kin of minor participants.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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RESEARCH ARTICLE

Lactate Dehydrogenase in Hypertensive Disorders in Pregnancy: Severity or Diagnosis Marker?

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Abstract

Background: Lactate dehydrogenase has had an exciting journey as a utility marker in different illnesses, but currently, its clinical utility has been relegated to confirm hemolysis, as a tumor marker, and as a diagnostic biomarker of preeclampsia. The findings of lactate dehydrogenase concentrations taking reference values to healthy persons are not consistent when these are related to hypertensive disorders in pregnancy, mainly to begin symptoms or little severity presentation. The goal in this work was to evaluate the maternal serum concentration of lactate dehydrogenase and its utility as a severity or diagnosis marker for hypertensive disorders in pregnancy.

Methods: In this retrospective study, we included 5,558 cases of HDP and 800 healthy pregnancies. HDP classification and LD values were collected from the medical records in the paper chart.

Results: The prevalence of HDP in our hospital was approximately 6.4 ± 0.1%. We found a tendency toward increases in median LD concentrations with the increasing severity of HDP and found a positive correlation ($p = 0.037$) or error probability of 0.037% between LD concentrations and severity of HDP in Mexican pregnant women.

Conclusion: Serum LD concentration in HDP is a marker of severity, diagnosis and adverse maternal outcomes.

Keywords

Lactate dehydrogenase, Hypertensive disorders in pregnancy, Diagnostic markers

Introduction

Lactate dehydrogenase (LD) is a cytoplasmic enzyme that is widely expressed in tissues and cells. LD is an enzyme in the glycolytic pathway catalyzes the oxidation of L-lactate to pyruvate with the mediation of nicotinamide adenine dinucleotide (NAD+) as the hydrogen acceptor. This reaction is reversible and can be detected in the laboratory in serum samples by measuring LD activity in terms of the rate of dihydronicotinamide adenine dinucleotide dehydrogenase (NADH) production

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determined spectrophotometrically at 340 nm [1,2]. LD is a critical serologic marker for diagnosis, staging/prognosis, and recurrence, and monitoring of germ cell tumors [3], as well as for multiple myeloma, another malignant disease wherein high LD levels are associated with disease severity and poor prognosis [4,5]. Serum LD levels increase in proportion to the clinical severity of idiopathic pulmonary arterial hypertension and have a strong, independent association with the long-term mortality of these patients. Assessing the potential role of LD as a biomarker and mediator involved in the pathogenesis of idiopathic arterial hypertension might be worthwhile [6]. LD has had an exciting journey as a utility marker in different illnesses, but currently, its clinical utility has been relegated to confirm hemolysis, as a tumor marker, and as a diagnostic biomarker of preeclampsia (PE) [3,7]. However, the findings of LD concentrations taking reference values to healthy persons are not consistent when these are related to hypertensive disorders in pregnancy (HDP), mainly to begin symptoms or mild PE.

The HDP are among the leading causes of maternal and perinatal morbidity and mortality worldwide [8]. The public classification system was adopted by the National High Blood Pressure Education Program (NHBPEP) Working Group in 1990 and subsequently endorsed by 46 medical organizations. The updated version in 2000 has become a standard that the American College of Obstetrics and Gynecology (ACOG) follows. From the NHBPEP original reports, guidelines from international societies have emerged, each one with their evidence, although many with similar recommendations [9]. The HDP should be classified as pre-existing hypertension, gestational hypertension, preeclampsia, or others hypertensive effects based on different diagnostic and therapeutic considerations. Hypertension in pregnancy is defined by systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg [10].

Dramatic changes in the cardiovascular system occur throughout gestation beginning soon after conception, presumably with the objective of increasing blood flow and nutrient delivery to the fetal-placental unit. The Healthy pregnancy is associated with increased endothelium-mediated relaxation, blunted response to vasoconstrictors, and increased flow-mediated dilation [11]. Modification of the placental bed arteries to reach a high-flow, low-resistance status to support this increased blood flow is achieved by extravillous trophoblast-mediated remodeling of spiral arteries, with a replacement of the endothelium by trophoblasts [12]. The link between abnormalities in trophoblast invasion and generalized maternal endothelial dysfunction seen in HDP, particularly in preeclampsia, maybe via release of placental factors, such as syncytial knots, shedding of syncytiotrophoblast basement membrane fragments (STBM), leukocyte and platelet membrane particles, activated neutrophils, cytokines, growth factors, angiogenic factors, and hormones [13]. These factors will interact with the maternal vascular endothelium, which may already be damaged and can cause maternal endothelial cell damages. The STBM may also damage the endothelium and activate neutrophils, and this may lead to endothelial dysfunction as part of the widespread intravascular inflammation [14]. Evidence for endothelial dysfunction in preeclampsia includes reduced *in-vitro* endothelium-dependent dilatation of isolated vessels, increased vascular reactivity in response to vasoconstrictor stimuli, and elevated biomarker levels associated with endothelial activation and injury [12]. Detection of high-risk patients with increased LD levels, as a marker of endothelial damage by HDP, mandates close monitoring and correct management to decrease both maternal and fetal morbidities [15]. In the present study, we evaluated the maternal serum concentration of LD and its utility as severity or diagnosis marker for HDP.

Material and Methods

In this retrospective study of 10 years, we included all women diagnosed with any HDP who were admitted to the Gynecology and Obstetrics Department of the Hospital General de Durango, Mexico, between January 2008 and December 2017.

Pregnant women with any HDP were identified by final diagnosis of the patient at discharge in the hospital and recruited by the archive and statistic department in agreement of the ICD-10 code. Patients with HDP diagnosed from localities outside the Durango state and women with HDP associated with trophoblastic disease were excluded. HDP diagnosis was confirmed by medical record review by the principal investigators. Gestational hypertension was defined by systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg on at least two occasions 6 hours apart, without proteinuria. Preeclampsia was defined as > 20 weeks gestation with incident hypertension (defined as a systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure 90 mmHg on at least two occasions 6 hours apart) and proteinuria (300 mg protein excreted over 24 h, or 30 mg/dL in a random urine sample or 1+ protein on urine dipstick). Eclampsia was defined by seizure occurrence in women with preeclampsia that cannot be attributed to other causes. The hemolysis, elevated liver enzymes, and low platelet (HELLP) syndrome in preeclamptic or eclamptic women was defined by platelet count less than 100,000 cells/mm^3, liver enzymes more than twice the normal value, and the presence of microangiophatic haemolytic anemia, or observation of burr cell schistocytes and polychromasia on peripheral blood smear observation. Healthy pregnancies were defined as those normotense pregnant women without complications before, during and later pregnancy resolution.

Maternal demographic data including place of birth, age, gravity, gestational age at delivery, mean arterial pressure (MAP), HDP classification, and LD values were

collected from the medical records in the paper chart. LD values were taken from the first laboratory examination during admission. The LD concentrations were determined through a dry chemistry method in Johnson & Johnson Vitros® 5.1 FS analyzer by (Ortho Clinical Diagnostics 1001 U.S 202 Raritan, NJ 08869), validated with daily internal quality control and monthly by external quality assurance programs.

Statistical analysis

The SPSS software (version 15.0; SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis; the clinical characteristics of the HDP sub-types were expressed as mean ± standard deviations (SD) or median and interquartile range (IQR). The mean of the continuous values was compared using the Student's t-test after testing for normality using the Kolmogorov-Smirnov test. A p-value equal to less than 0.05 was considered statistically significant. Mann-Whitney U-test, or student's t-test depending on the normality distribution, was performed to compare the groups. To calculate bi-variate correlation between LD values and HDP severity expressed in the probability that LD concentrations are a severity marker of HDP, we calculated the Spearman range correlation, and data were represented in graphic distribution by error bars later. Finally, a baseline was obtained with IQR (Q1 - Q3) of LD values for each HDP classification, and reference values were established for each classification.

Results

Ethical approval was obtained from the Institutional Review Boards of the Hospital where this retrospective study was conducted. In our hospital 107,937 patients were attended in the gynecological & obstetrical service during 10 years, and 86,202 deliveries occurred during the same period, including fetal deaths 20 weeks gestation. A total of 5,552 women presented with pregnancy complicated with any HDP category in agreement with the criteria of NHBPEP and Technical Guidelines for Diagnosis, Prevention and Management of Preeclampsia-Eclampsia of Health Ministry in Mexico. The case distribution in 2008 to 2017 with the different sub-classifications of HDP, including 800 healthy pregnancies, is shown in Table 1. All cases were confirmed after the medical records were reviewed. The prevalence of HDP

Table 1: Lactic dehydrogenase values for different hypertensive disorders of pregnancy including normo-evolutive pregnancy.

Hypertensive disorders of pregnancy	Cases (n)	LD concentration (IU/L)	Standard deviation	Q1 Minimum value (IU/L)	Q3 Maximum Value (IU/L)
Normo-evolutive pregnancy	800	274.49§	101	201	360
Gestational hypertension	2,057	515.05¢	339	400	565
Mild preeclampsia	1,089	537.13§	122	463	567
Severe preeclampsia	1,817	654.92§	222	522	729
Eclampsia	172	747.56§	219	556	921
Severe preeclampsia + HELLP	361	1,492.4¢	1,178	790	2,066
Eclampsia + HELLP	56	4,634.79¢	3,855	2,117	4,898

§Media; ¢Median.

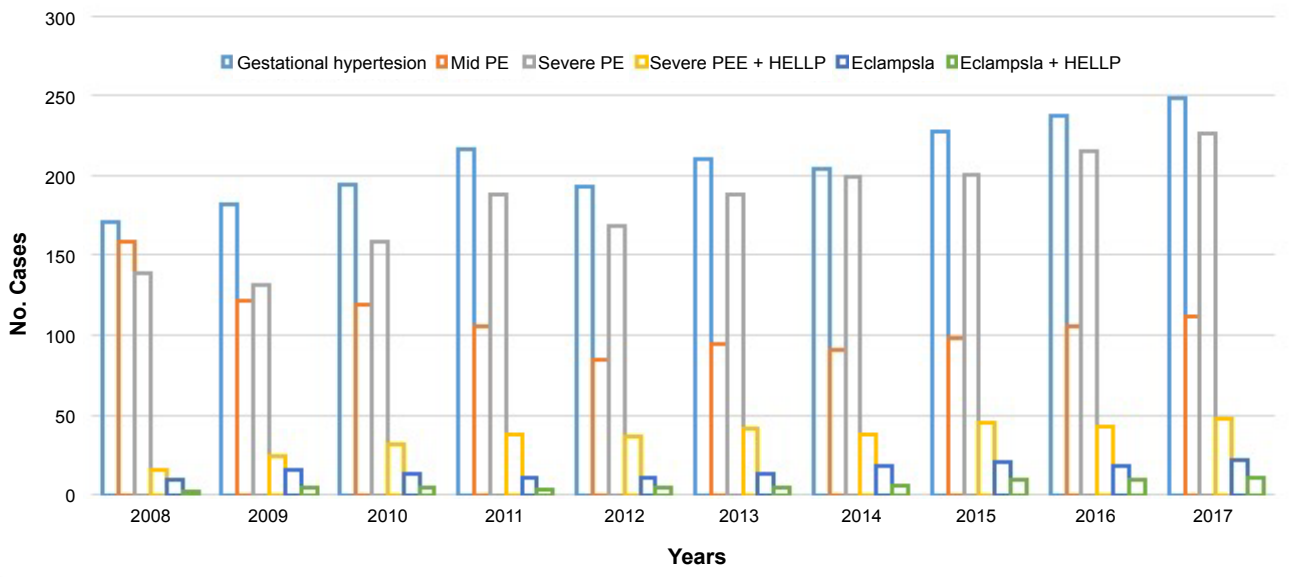

Figure 1: Cases of Hypertensive disorders of pregnancy from 2008 to 2017.

in our hospital was about 6.4 ± 0.1%. The occurrence of HDP has increased each year in proportion with the pregnant women attended. However, the proportion related to HDP severity had been changed with respect to mild PE and severe PE because severe PE had been increasing, whereas mild PE had been decreasing over these 10 years possibly by changes in clinical criterial for classification of mild and severe preeclampsia; however, gestational hypertension and eclampsia remained stable (Figure 1). With respect to the frequency of HDP: Gestational hypertension 37.1% (n = 2,057), mild PE 19.6% (n = 1,089), severe PE 32.7% (n = 1,817), eclampsia 3.1% (n = 172), severe PEE with HELLP 6.4% (n = 361), and eclampsia + HELLP only presented, 1.01% (n = 56), of all cases, respectively. The mean of the chronologic age of women was 24.8 ± 7.3 years; the mean for gestational age was 36.7 ± 4.0 weeks. The mean number of pregnancies was 2.29 ± 1.6, and MAP was 108.8 ± 18.3 mmHg. With respect to the chronological age of pregnant women, those who had eclampsia had the lowest mean age (21.6 years ± 6.2 SD), compared with media the total of the women.

To calculate the LD reference concentrations for HDP; we include 14.5% (n = 800) LD values of healthy pregnancies which are shown in Table 1. So, LD reference values were stablished for normo-evolutive pregnancies and pregnancies complicated with HDP. Then, we found a tendency to increase the mean of LD concentrations in relation to HDP severity and PE complicated with HELLP syndrome. After establishing the data of non-parametric distribution, and Spearman range correlation analysis, we found a correlation (p = 0.037) or error probability of 0.037% between LD concentrations and HDP severity in Mexican pregnant women (Figure 2). Likewise, the median and interquartile ranges (IQR) were established for LD values to find reference ranges through Q1 and Q3 values and, propose a new reference range for Mexican normo-evolutive pregnant women and Mexican pregnant women complicate with HDP (Table 1). Finally we established a baseline of LD values related to HDP (Figure 3).

Discussion

Based on the World Health Organization, HDP has been the second leading cause of maternal death globally to date [16], but it is the leading cause of maternal death in Latin America with up to 25% cases [17,18]. The most at risk age groups are young mothers aged between 10 and 24 years, and there are groups that present HDP with more severity [19]. Our population studied had a similar risk, with more prevalence of severe PE and eclampsia in young women (21.6 ± 6.2 years). In contrast, change in the decrease of frequency for mild PE and increase for severe PE was found in

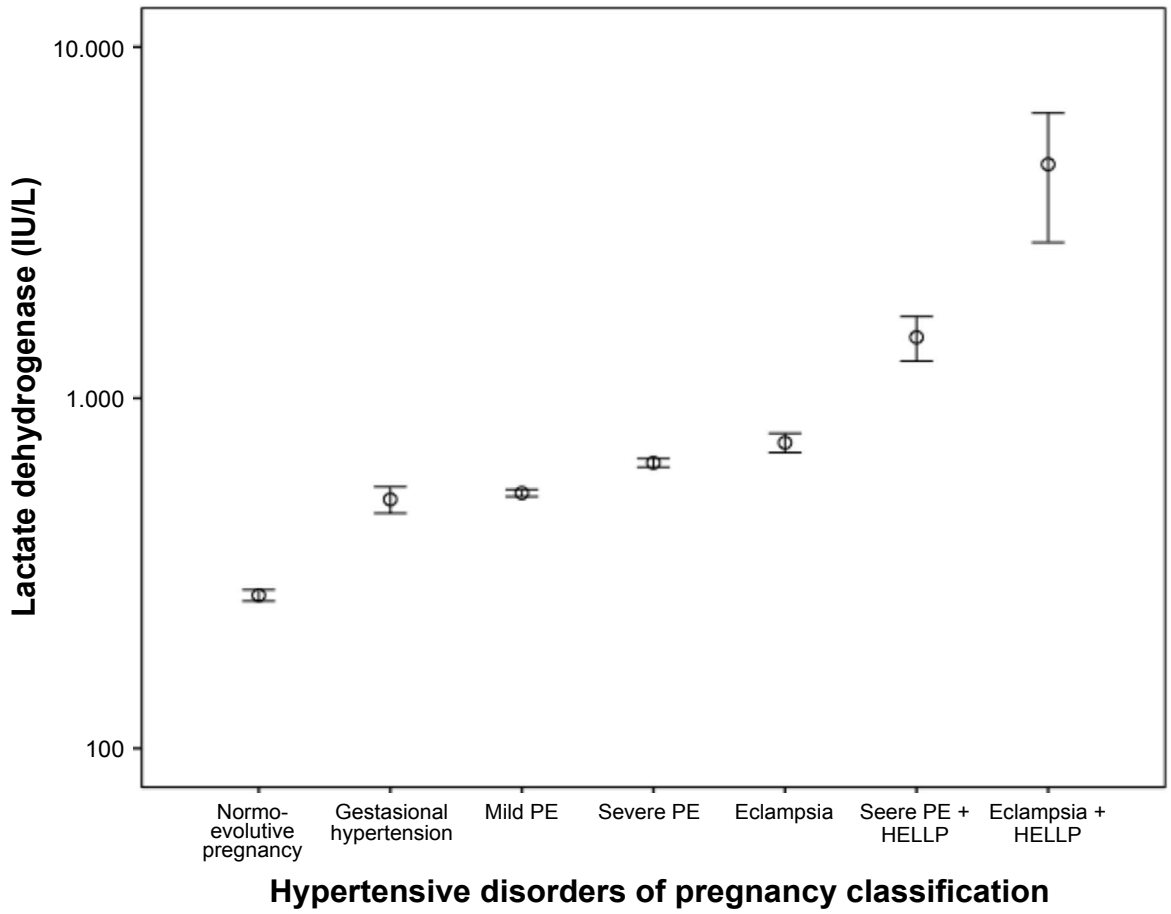

Figure 2: Correlation between HDP and LD concentrations.

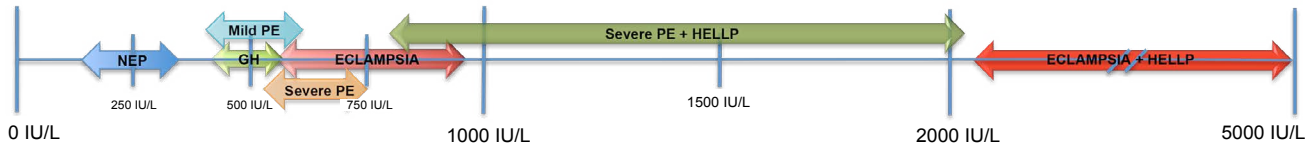

Figure 3: Baseline concentrations of LD in Mexican pregnant women with normo-evolutive pregnancy and HDP.

this study, and similar findings were reported first by Kuklina, et al. [20] in 2006, and recently by Cavazos-Rehg, et al. [21]. We also found that eclampsia was more frequent in first-time pregnant women unlike in any other HDP cases. The goal of monitoring symptoms and biochemical markers in patients with HDP is to appropriately time interventions and delivery while avoiding untoward maternal or neonatal complications. In contrast, LD has had an interesting journey, but today its clinical utility has been relegated to confirm hemolysis and serve as a tumor marker [22]. A study similar to ours was conducted by Qublan, et al. [15] and those authors found elevated LD levels at 111 IU/L in women with mild and severe PE, classifying three groups and LD values as follows: healthy pregnant (600 IU/L), mild PE (600-800 IU/L), and severe PE (800 IU/L), and they concluded that LD is indicative of cellular damage and dysfunction, and it can be used as a biochemical marker because it reflects the severity of the disease, occurrence of complications, and fetal outcome. In contrast, Jaiswar, et al. [22] conducted a study to evaluate LD as a biochemical marker for PE-eclampsia, and they analyzed 146 Indian women. They reported LD levels of 278.3 ± 119.2 IU/L in healthy pregnant women, and this value is similar to that found in our study (n = 379 and LD = 274 ± 100.7 IU/L). Furthermore, they concluded that LD levels had a significant association with various maternal and fetal outcomes in patients with PE and eclampsia. Other biomarkers in serum, such as soluble fms-like tyrosine kinase-1 (sFLt-1), vascular endothelial growth factor (VEGF), soluble intracellular adhesion molecule-1 (sICAM-1) and, soluble vascular intracellular adhesion molecule-1 svICAM-1, were varied and could not be pooled. To date, the literature on serum biomarker involvement in angiogenesis or inflammation after HDP has been inconsistent, with reports of lower [23] or higher [24] levels in women with PE. In fact, controversy exists because while Myatt, et al. [25] did not find that assessment of changes in angiogenic markers alone from the first or second trimester can improve predictive power, Widmer, et al. [26] also concluded in a multicenter study that angiogenic biomarker tests performed at ≤ 20 weeks gestation did not perform well enough in predicting PE for incorporation into current practice. However, these serum biomarkers, which are responsible for the initial endothelial insult during early pregnancy and are more expressed in placental tissue than in the endothelium, may not be detected at elevated levels after the index pregnancy. To clarify the confusing data regarding angiogenic factors and the need to obtain a better interpretation of traditional biomarkers, we propose LD values as indicative of cellular damage and endothelial dysfunction in HDP, and LD can be used as an effective biochemical marker because it reflects the severity of the disease, occurrence of complications, and fetal outcomes [15] in the Mexican population.

HDP diagnosis and classification are the main goals in pregnant women with high blood pressure, and the correct evaluation of clinical and laboratory parameters is important to achieve these goals. In this study, we showed that LD concentrations in HDP are predictive of adverse maternal outcomes and, we propose new LD reference values to classify HDP based on serum LD concentration and established a baseline of LD concentrations based on HDP severity. Currently, the basic biochemical tests for LD remain in effect to diagnose and classify HDP, and their use continues to provide valuable information for diagnosis and classification of pregnant women with HDP.

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Disclosure

No potential conflict of interest was reported by the authors.

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Original Article

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Human Papillomavirus Infection in Female Sex Workers: A Case Control Study

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Abstract

Background: To determine the association of infection with human papillomavirus (HPV) and the occupation of female sex worker; and the correlation of infection with HPV with sociodemographic, clinical and behavioral characteristics of female sex workers.

Methods: We performed a case-control study of 217 female sex workers and 354 women without sex work in Durango City, Mexico. We determined the prevalence of infection with HPV in cervical samples of women using polymerase chain reaction, and HPV genotypes were determined using line probe assay. Bivariate and multivariate analyses were used to assess the association between the characteristics of women and infection.

Results: Twelve (5.5%) of the 217 sex workers, and 10 (2.8%) of the 354 control women were positive for HPV DNA (age-adjusted OR = 1.51; 95% CI: 0.62 - 3.68; P = 0.36). Six (50.0%) of the 12 HPV DNA positive sex workers had infections with high-risk genotypes (16, 31, 33, 35, 51, 58). Seven (70%) of the 10 HPV DNA positive control women had infections with high-risk genotypes (16, 18, 56, 58, and 66). The frequency of high risk genotypes in the control women was equal with that found in the female sex workers (P = 0.41). Logistic regression analysis showed that the variable alcohol consumption was associated with HPV infection (OR = 4.0; 95% CI: 1.0 - 16.0; P = 0.04).

Conclusions: No association between HPV infection and female sex work was found in our setting. High risk HPV genotypes were prevalent among the women studied. Results can be used for the design of preventive measures against HPV infection.

Keywords: Human papillomavirus; Prevalence; Female sex workers; Case-control study

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Introduction

Human papillomavirus (HPV) is a double-stranded DNA virus causing infections in humans [1]. Epithelial cells of the skin and mucosa are targets for infection with this virus [2]. Infection with HPV may lead to cervical and oropharyngeal cancers [3]. Cervical cancer is the third most common malignancy in women and persistent infection with HPV is the major risk factor for the development of this disease [4]. There are over 200 HPV genotypes, but only a subset of HPV variants so called "high-risk" HPV genotypes cause cervical disease [5]. Fourteen HPV genotypes are considered high risk types including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 [6, 7]. HPV 16 and 18 genotypes are the most common types [7]. HPV is transmitted by direct contact [8], usually by sexual intercourse [9]. HPV causes the most common sexually transmitted infection [10]. Female sex workers have a high prevalence of infection with high risk HPV genotypes [11]. These workers constitute an epidemiologically important population group for HPV infection because they may acquire this infection and transmit it to other sexual partners.

The epidemiology of HPV infection in female sex workers in Mexico has been poorly studied. In a previous study of female sex workers in the northern Mexican city of Gomez-Palacio in Durango State, researchers found a 28.57% prevalence of HPV infection in 168 sex workers studied using polymerase chain reaction (PCR) [12]. In the present study, that was performed in another city (the capital) of Durango State, and with a different study design (case-control) from the previous study (cross-sectional), we aimed to determine: 1) The association between the occupation of female sex work and

infection with HPV in the northern Mexican city of Durango; 2) The HPV genotypes infecting the women studied; and 3) The HPV prevalence association with behavioral factors of the female sex workers studied.

Materials and Methods

Study design

We performed a case-control study. Cases were female sex workers, and controls were women without the occupation of sex worker. Controls were matched with cases by sex (females). This study was performed from November 2014 to May 2016.

Description of the population groups studied

Cases included female sex workers registered at the Municipal Clinic for Sanitary Inspection in Durango City, Mexico. All registered female sex workers were invited to participate. A female sex worker was defined as a woman working at the sex industry. Inclusion criteria for enrollment of cases were: 1) Registered female sex worker; 2) With at least 1 year of working in the sex activity; and 3) Aged 18 years and older.

Controls included sexually active women but without sex work attended at the Clinic for Family Planning in the Institute for Scientific Research of the Juarez University of Durango State in Durango City, Mexico.

Sociodemographic, obstetric and behavioral data of female sex workers

A questionnaire was used to record the sociodemographic, obstetric and behavioral data of female sex workers. Items about age, education, and obstetric data as pregnancies, deliveries, cesarean sections, and miscarriages were included in the questionnaire. In addition, behavioral factors as duration in the activity (years), condom use, contact with semen during vaginal intercourse, practice of oral or anal sex, area of work, sex activity in Mexican States other than Durango or abroad, injuries during sex activity, alcohol or tobacco consumption, and drug use from the female sex workers were obtained.

Laboratory tests

A cervical swap sample was obtained from each participant. Samples were kept in 2SP medium and stored at -70 °C until analysis. DNA of samples was extracted by proteinase K digestion, and phenol-chloroform extraction. Detection of HPV DNA was performed by PCR using the primers MY09/11. The amplification mixture contained 2 mM $MgCl_2$, 0.2 µM of primers, 2 units of Taq polymerase, 0.2 mM of dNTPs, 2 ng of DNA, and buffer in a 50 µL reaction volume. Amplifications were performed for 39 cycles with the following cycling parameters: 94 °C for 1 min, 55 °C for 2 min, and 72 °C for 2.5 min. PCR products were run in 2% agarose gels and stained with ethidium bromide.

Genotyping of HPV DNA was performed by a commercially available line probe assay kit: INNO-LiPA HPV Genotyping Extra (INNOGENETICS N.V. Gent, Belgium). This test is based on the principle of reverse hybridization and allows the detection of 28 different HPV genotypes. This genotyping assay uses biotinylated primers (SPF10) to amplify the L1 region of HPV. Amplicons are denatured and hybridized with specific oligonucleotide probes on membrane strips. Streptavidin-conjugated alkaline phosphatase was added and incubation with BCIP/NBT chromogen yielded a purple precipitate. Results were interpreted visually. All assays were performed following the instructions of the manufacturer.

Statistical analysis

The statistical analysis was performed with the software SPSS for Windows version 15.0 (SPSS Inc. Chicago, Illinois) and Epi Info 7. Calculation of the sample size was performed using the following data: 95% two-sided confidence level, a power of 80%, a 1:1.5 ratio of cases and controls, a reference seroprevalence of 16.67% [13] as the percentage outcome in unexposed group, and an odds ratio (OR) of 2.0. The result of the sample size calculation was 172 cases and 258 controls. Descriptive statistics were used to calculate the prevalence of HPV infection. The risk of HPV infection was calculated by the OR with 95% confidence interval (CI) adjusted by age. The association between HPV infection and the characteristics of female sex workers was assessed in available data by the Pearson's Chi-squared test and the Fisher exact test (when values were small). Characteristics of female sex workers with a P value ≤ 0.20 obtained by bivariate analysis were selected for multivariate analysis. ORs and 95% CIs were calculated by logistic regression with the Enter method. A P value < 0.05 was considered statistically significant.

Ethics approval

This project was approved by the Ethics Committee of the Institute for Scientific Research of Juarez University of Durango State, Mexico. Participation in this study was voluntary, and a written informed consent was obtained from all participants.

Results

A total of 571 women were included in the study. Of them, 217 were sex workers and 354 were control subjects. Sex workers were 32.4 ± 9.9 (range: 18 - 67) years old. Whereas control women were 36.4 ± 10.0 (range: 18 - 67) years old. Control women were significantly older than sex workers ($P < 0.01$). Therefore, statistical analysis was performed with age adjustment. Of the 217 sex workers, 12 (5.5%) were positive for HPV

Table 1. Correlation of HPV Infection and General Sociodemographic and Obstetric Characteristics of Female Sex Workers and Controls

Characteristic	Sex workers			Controls			P value
	Subjects tested	Prevalence of HPV infection		Subjects tested	Prevalence of HPV infection		
	No.*	No.	%	No.*	No.	%	
Age (years old)							
20 or less	17	2	11.8	10	1	10	1
21 - 30	84	7	8.3	105	6	5.7	0.48
31 - 40	64	1	1.6	112	1	0.9	1
41 or more	45	2	4.4	127	2	1.6	0.28
Educational level							
No education	4	0	0	0	0	0	-
1 - 6 years	59	4	6.8	15	0	0	0.57
7 - 12 years	136	8	5.9	163	6	3,7	0.37
> 12 years	11	0	0	170	3	1.8	1
Pregnancies							
Yes	188	11	5.9	302	7	2.3	0.04
No	19	1	5.3	52	3	5.8	1
Deliveries							
Yes	136	9	6.6	211	6	2.8	0.09
No	62	2	3.2	132	4	3	1
Cesarean sections							
Yes	78	3	3.8	119	2	1.7	0.38
No	118	8	6,8	211	8	3,8	0.28
Abortions							
Yes	50	0	0	83	1	1.2	1
No	144	11	7.6	246	9	3.7	0.08

*Sums may not add up to 217 cases or 354 controls because of some missing values.

DNA detection by PCR. Whereas of the 354 control women, 10 (2.8%) were positive for PCR. No statistically significant difference in the frequencies of HPV DNA detection between sex workers and control subjects was found (age-adjusted OR = 1.65; 95% CI: 0.69 - 3.96; P = 0.25).

Of the 12 HPV DNA positive female sex workers, eight had single infections and four double or triple infections. HPV genotypes found in the eight sex workers with single infections were: 6, 11, 16, 26, 35, 40, 43, and 51. HPV genotypes found in the four sex workers with multiple infections were: 69 and 71 in two; 31, 40, and 58 in one; and 31, 33, and 53 in one. Six (50.0%) of the 12 HPV DNA positive sex workers had infections with high-risk genotypes (16, 31, 33, 35, 51, 58); whereas of the 10 HPV positive control women, eight had infection with a single HPV genotype, and two had infections with two or three HPV genotypes. Single HPV genotypes found in eight HPV DNA positive control women were: genotype 16 in two, genotype 18 in two, genotype 66 in one, and genotype 40 in three. HPV genotypes found in the two control women with double or triple infections were genotypes 56 and 58 in one, and genotypes 31, 40, and 58 in the other one. Seven (70%) of these 10 control women had infections with high-risk genotypes (16, 18, 56, 58, and 66). The frequency of high-risk genotypes in the control women was equal to that found in the female sex workers (P = 0.41).

Stratification of socioeconomic and obstetric characteristics including age, educational level, and history of pregnancies, deliveries, cesarean sections, and miscarriages showed a similar (P > 0.05) rate of HPV positivity in female sex workers and controls (Table 1). None of these characteristics was associated with HPV infection. Of the behavioral data (Table 2), the variables time of sexual activity (≤ 5 years), sex activity in Mexican States other than Durango and alcohol consumption had P values ≤ 0.20 by bivariate analysis and were selected for multivariate analysis. Other behavioral characteristics including condom use, contact with semen during vaginal intercourse, practice of oral or anal sex, area of work, sex activity abroad, injuries during sex activity, tobacco consumption, and drug use had P values > 0.20 by bivariate analysis. Results of the logistic regression analysis are shown in Table 3. Only the variable alcohol consumption was associated with HPV infection (OR = 4.0; 95% CI: 1.0 - 16.0; P = 0.04).

Table 2. Results of Bivariate Analysis of Behavioral Characteristics of Female Sex Workers and Infection With HPV

Characteristic	Subjects tested	Prevalence of HPV infection		P value
	No.*	No.	%	
Time of sexual activity (years)				
1 to 5	25	3	12	0.07
6 to 11	51	5	9.8	
11 or more	134	4	3	
Condom use				
Yes	119	8	6.7	0.54
No	85	4	4.7	
Contact with semen during vaginal intercourse				
Yes	90	5	5.6	0.9
No	118	7	5.9	
Practice of oral sex				
Yes	126	5	4	0.22
No	83	7	8.4	
Practice of anal sex				
Yes	18	0	0	0.6
No	190	12	6.3	
Area of work				
Urban	205	12	5.9	0.91
Suburban	2	0	0	
Rural	1	0	0	
Sex activity in Mexican states other than Durango				
Yes	59	1	1.7	0.18
No	150	11	7.3	
Sex activity abroad				
Yes	4	0	0	
No	206	12	5.8	
Injuries during sex activity				
Yes	13	0	0	1
No	195	12	6.2	
Alcohol consumption				
Yes	36	4	11.1	0.11
No	181	8	4.4	
Tobacco consumption				
Yes	42	1	2.4	0.46
No	175	11	6.3	
Drug use				
Yes	13	1	7.7	0.53
No	204	11	5.4	

*Sums may not add up to 217 because of some missing values.

Table 3. Results of the Multivariate Analysis of a Selection of Characteristics of Female Sex Workers and Infection With HPV.

Characteristic	Odds ratio	95% Confidence interval	P value
Alcohol consumption	4	1.0 - 16.0	0.04
Time of sexual activity (≤ 5 years)	3.4	0.79 - 15.3	0.09
Sex activity in Mexican states other than Durango	0.18	0.02 - 1.5	0.11

Discussion

There is scanty information about the epidemiology of HPV infection in female sex workers in Mexico. Therefore, we sought to determine the association of HPV infection and female sex work in Durango City, Mexico. Comparison of the prevalence of HPV infection obtained by PCR indicates that female sex workers had a similar prevalence of HPV infection to control women. This result thus suggests that female sex workers do not have an increased risk of HPV infection. This finding is unexpected since having multiple sexual partners may lead to higher HPV transmission, and in a review of 35 studies, researchers found that female sex workers had a high prevalence of HPV infection [11]. In a study in Antwerp, Belgium, researchers found a higher prevalence of high-risk HPV infection in female sex workers than in controls of the general population [14]. Similarly, in a study in Hungary, higher prevalences of HPV infection and high-risk HPV infection were found in female sex workers than in control women [15]. Furthermore, in a study in Japan, researchers found a higher prevalence of intermediate- and high-risk HPV infection in commercial sex workers than in control subjects [16]. On the other hand, the lack of association between HPV infection and sex work found in our study agrees with a similar result found in a study of commercial sex workers in Australia [17]. In a national context, we are aware of only two previous reports on the epidemiology of HPV infection in female sex workers in Mexico. In a previous descriptive study of female sex workers in the northern Mexican city of Gomez-Palacio in Durango State, researchers found a high (28.57%) frequency of HPV infection in 168 sex workers who have practiced prostitution for more than 5 years [12]. In a study of female sex workers from Mexico City, high prevalences of HPV infection (48.9%), and of high-risk HPV genotypes (43%) were found [18]. The prevalence of HPV infection found in our study is thus much lower than those reported in previous studies in female sex workers in Mexico. It is not clear why female sex workers in Durango City had lower prevalence of HPV infection than those in Gomez-Palacio City and Mexico City. It is likely that differences in the sociodemographic or behavioral characteristics of sex workers including age, duration in the activity, condom use, or number of sexual partners might explain the differences in prevalences among the studies. On the other hand, we compared the prevalence of HPV infection found in sex workers from Durango City to those reported in women in the region. The low prevalence of HPV infection in sex workers found in our study is comparable to the 4.8% prevalence reported in women seeking for cervical Papanicolaou examination in three public health centers in Durango City [19], and the 2.15% prevalence found in women with normal cyto-histopathologic diagnosis in Gomez-Palacio City [13].

The frequency of high-risk HPV genotypes found in our study (50%) is higher than the 11.90% prevalence of high-risk HPV genotypes reported in female sex workers in Gomez-Palacio City, Durango [12], and comparable to the 43% prevalence reported in sex workers from Mexico City [18].

We found that the variable alcohol consumption was associated with HPV infection in female sex workers. To the best of our knowledge, there is no previous report on the association between female sex work and alcohol consumption. It is possible that sex workers with alcohol consumption take less care of preventive measures against HPV infection. Substance use including alcohol consumption has been linked to HPV infection in male sex workers in Vietnam [20].

Conclusions

We conclude that HPV infection is not associated with female sex work in Durango City. Female sex workers in Durango City have a low prevalence of HPV infection but a high prevalence of high-risk HPV genotypes. The association between HPV infection and alcohol consumption deserves further investigation.

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Conflict of Interest

No competing interests exist.

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ORIGINAL ARTICLE

Lack of Association between Mannose-binding Lectin 2 Codons 54 and 57 Gene Polymorphisms and Cervicovaginal Infections in Mexican Women

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ABSTRACT

The mannose-binding lectin (MBL) 2 gene has an important function in the innate immune response and activation of the third pathway of the complement system. Some studies have assessed the association of the MBL2 gene polymorphisms with cervicovaginal infections (CVI); however, there is no information about this association in Mexican women. This study aimed to determine the association between the MBL2 codons 54 and 57 gene polymorphisms with CVI in a sample of Mexican women. Through a cross-sectional study, blood samples and cervicovaginal cultures were obtain from 354 women. MBL2 genotyping was performed by real-time polymerase chain reaction with Taqman probes. Of the 354 women studied, 128 (36.2%) had CVI and 226 (63.8%) were healthy. The frequencies of the C and T variants in codon 54 in women with CVI were 83% and 17%, respectively; whereas the frequencies of these variants in healthy women were 82% and 18%, respectively. The frequencies of variants C/C, C/T, and T/T in women with CVI were 68%, 31%, and 1%, respectively; whereas the frequencies of these variants in healthy women were 68%, 29%, and 3%, respectively. With respect to codon 57, the frequencies of variants C and T were identical in women with CVI and in healthy women (97% and 3%, respectively). The frequencies of variants C/C, C/T, and T/T were identical in women with CVI and in healthy women (94%, 6%, and 0%, respectively). We conclude that MBL2 codons 54 and 57 gene polymorphisms do not associate with CVI in Mexican women. (*Int J Biomed Sci* 2017; 13 (2): 79-83)

Key words: Cervicovaginal infections; MBL2 gene; codon 54; codon 57

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INTRODUCTION

Cervicovaginal infections (CVI) are a group of gynecological entities characterized by replacement of normal vaginal flora with infectious agents including virus, bacteria, fungi and protozoa (1, 2). CVI occur in women of any age and are one of the most important causes of medical consultations in primary healthcare centers (3, 4). It is estimated that 90% of CVI are caused by three groups of pathogens: a) anaerobic bacteria, mainly *Gardnerella vaginalis* leading to bacterial vaginosis; b) yeasts of the *Candida spp* genus leading to vulvovaginal candidiasis; and c) the protozoan parasite *Trichomonas vaginalis* (5-7). The innate immune system represents the first line of defense against infectious agents leading to an immediate response through several effector mechanisms that recognize and remove pathogens, and activate the adaptive immune system (8, 9). The mannose-binding lectin (*MBL*) 2 is a protein codified by a gene located on chromosome 10q11.2 (10, 11). This serum lectin is synthetized by the liver and it is released to the blood stream during the innate immune response against virus, bacteria, yeasts, and parasites (12). The *MBL* binds cell surface carbohydrates of pathogens mediating opsonization either directly or through complement activation by the lectin pathway (13). Low or deficient concentrations of MBL in serum are mainly due to single nucleotide polymorphisms of exon 1 of MBL2 gene (14). MBL2 codons 54 and 57 gene polymorphisms (variant allele O; wild-type allele designated as A) are denoted as B and C, respectively (15). These point mutations result in amino acid substitutions in the collagen region: in codon 54 (GGC->GAC, Gly->Asp, allele B), and in codon 57 (GGA->GAA, Gly->Glu, allele C) (16-18). Several studies have reported the association of MBL2 polymorphism with CVI (19-21). However, there is not any report about this association in Mexican population.

MATERIALS AND METHODS

Selection and description of participants

Through a cross-sectional study, 354 women attending consultations in the Family Healthcare Department in the Institute for Scientific Research of the Juárez University of Durango State in Durango City, Mexico were examined. Women were enrolled in the study from November 2014 to June 2016. Inclusion criteria for enrollment were: 1) age 17 years and older; 2) sexually active; and 3) who voluntarily accepted to participate in the study. Exclusion criteria were: 1) pregnant women, 2) women during menstrual period; 3) women under treatment for CVI in the last 10 days (including vaginal ovules, creams, or douching); 4) women with a recent miscarriage or postpartum; 5) history of hysterectomy; 6) suffering from autoimmune or systemic diseases; and 7) under treatment with immunosuppressive agents or antibiotics.

Technical information

Cervicovaginal secretions from participants were obtained with sterile cotton swaps and placed into routine culture media (chocolate agar, blood agar, and Thayer-Martin agar). In addition, a direct microscopic examination of the cervicovaginal secretions for the presence of pathogens was performed. Cervicovaginal secretions were also tested for the presence of amines, and examined using the Gram stain.

DNA was obtained from whole blood of participants by the QIAamp DNA Blood Mini Kit (QIAGEN) following the instructions of the manufacturer. The yield DNA concentration and purity were measured by the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Germering, Germany). Genotyping was performed using a real-time PCR equipment (StepOne, Applied Biosystems, Carlsbad, CA, USA) with TaqMan probes (codon 54: rs1800450; codon 57: rs1800451; Life Technologies, Australia). Typical reactions to a final volume of 20 µl consisted of 10 ng of genomic DNA, 0.625 µl TaqMan SNP genotyping assay, and 5.0 µl of genotyping master mix. Amplification was performed at 60°C for 30 seconds and 95°C for 10 minutes followed by 40 cycles of 92°C for 15 seconds and 60°C for 1 minute, and a final step of 60°C for 30 seconds.

Ethics aspects

This project was approved by the Ethics Committee of the Institute for Scientific Research of the Juárez University of Durango State, Mexico. An informed consent was obtained from all participants.

Statistics

Statistical analysis was performed using the software SPSS version.15.0. Allelic, genotypic and haplotypic frequencies were calculated with the aid of the software SNPStats, and odds ratio (OR) and 95% confidence interval (CI) were calculated. *P* values less than 0.05 were considered statistically significant.

RESULTS

One hundred and twenty-eight (36.2%) of the 354 women studied had CVI. Of them, 72 (20.3%) had bacterial vag-

inosis, 49 (13.8%) vulvovaginal candidiasis, and 7 (2.0%) trichomoniasis. Two hundred and twenty-six (63.8%) women were healthy. Mean age of women was 36.4 ± 10.3 (range: 17-67) years. Mean age at first sexual relation was 19.3 ± 3.8 (range: 11-40) years. Mean number of sexual partners was 3.0 ± 4.2 (range: 1-50). Mean number of sexual intercourses a month was 6.9 ± 5.6 (range: 0-40), and the median number of miscarriages was 0 (range 0-7). The frequencies of the C and T variants in codon 54 in women with CVI were 83% and 17%, respectively; whereas the frequencies of these variants in healthy women were 82% and 18%, respectively. The frequencies of variants C/C, C/T, and T/T in women with CVI were 68%, 31%, and 1%, respectively; whereas the frequencies of these variants in healthy women were 68%, 29%, and 3%, respectively. No association between the C/C reference genotype, C/T genotype polymorphism (OR=0.98; 95% CI: 0.60-1.58), and T/T genotype (codominant, OR=4.65; 0.55-39.1) and CVI was found. Codon 54 polymorphisms had genotypic distributions consistent with Hardy-Weinberg equilibrium in women with CVI (P=0.19), and in healthy women (P=1.0). With respect to codon 57, the frequencies of variants C and T were identical in women with CVI and in healthy women (97% and 3%, respectively). The frequencies of variants C/C, C/T, and T/T were identical in women with CVI and in healthy women (94%, 6%, and 0%, respectively). No association between this polymorphism (C/C reference genotype, C/T genotype, OR=1.18; 95% CI: 0.45-3.09) and CVI was found. Codon 57 polymorphisms had genotypic distributions consistent with Hardy-Weinberg equilibrium in women with CVI (P=1.0), and in healthy women (P=1.0). A correlation of the allelic and genotypic frequencies and the clinical characteristics of the women studied is shown in Table 1. Results of the association analysis of codons 54 and 57 MBL2 haplotypes with CVI are shown in Table 2. No haplotypic association with CVI was found (P=0.74).

Table 1. Allelic and genotypic frequencies of codons 54 and 57 polymorphisms of MBL2 gene in the women studied

Diagnosis[a]	Codon	Genotype	Positive No. (%)	Allele	Positive No. (%)	P^b value
Vulvovaginal candidiasis (n=49)	MBL54	C, C	36 (73.5)	C	85 (86.7)	
		C, T	13 (26.5)			
		T, T	0	T	13 (13.3)	0.27
Bacterial vaginosis (n=72)	MBL54	C, C	47 (65.3)	C	118 (81.9)	
		C, T	24 (33.3)			
		T, T	1 (1.4)	T	26 (18.1)	0.95
Trichomoniasis (n=7)	MBL54	C, C	5 (71.4)	C	12 (85.7)	
		C, T	2 (28.6)			
		T, T	0	T	2 (16.6)	1.00
Controls (n=230)	MBL54	C, C	155 (67.4)	C	378 (82.1)	
		C, T	68 (29.6)			
		T, T	7 (3.0)	T	82 (17.8)	
Vulvovaginal candidiasis (n=49)	MBL57	C, C	47 (95.9)	C	96 (97.9)	
		C, T	2 (4.1)			
		T, T	0	T	2 (2.0)	1.00
Bacterial vaginosis (n=72)	MBL57	C, C	67 (93.1)	C	139 (96.5)	
		C, T	5 (6.9)			
		T, T	0	T	5 (3.4)	0.77
Trichomoniasis (n=7)	MBL57	C, C	7 (100.0)	C	14 (100.0)	
		C, T	0			
		T, T	0	T	0	1.00
Controls (n=230)	MBL57	C, C	217 (94.3)	C	447 (97.1)	
		C, T	13 (5.7)			
		T, T	0	T	13 (2.8)	

[a]Four women had more than one infection; [b]Compared to controls (Fisher exact test).

Table 2. Association between codons 54 and 57 haplotypes of MBL2 gene and cervicovaginal infections

Codon 54	Codon 57	Women with cervicovaginal infections (n=124)[a]	Controls (n=230)	OR (95% CI)[b]	*P* value
C	C	81	79.8	-----	----
T	C	16.2	17.4	1.16 (0.73-1.83)	0.53
C	T	2.8	2.5	1.29 (0.47- 3.53)	0.62
T	T	0	0.3	[c]	

General haplotypic association: *P*=0.74. [a]Cervicovaginal infections: bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis; [b]Age-adjusted; [c]Undefined because of cells with a 0 value.

DISCUSSION

Cervicovaginal infections represent a public health problem in Mexican women. These infections have a high morbidity, and their complications are important cause of mortality in women at reproductive age (22). The present study aimed to identify the possible influence of codons 54 and 57 polymorphisms of *MBL2* gene on the presence of CVI including bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis in Mexican women.

Genetic components, vaginal microbiota, and local immunity play not only an important role in the health of women but also may contribute to a higher susceptibility to certain infections (23, 24). MBL2 is a vaginal component that protects against repeated proliferation of atypical vaginal microflora (25). Polymorphisms of the structural region of MBL2 gene, especially codon 54 and in a minor extent codon 57, cause alterations in the mannose-binding lectin production (11). Several studies in women populations have found a significant association between recurrent vaginal infections, especially vulvovaginal candidiasis and bacterial vaginosis, with the presence of codon 54 polymorphisms of MBL2 gene (7, 9, 14, 20, 21, 26). However, this association was not found in our study. It is important to mention that recurrent vulvovaginal infections were not considered in the present study. Only acute infections were included in our study. With respect to codon 54, the genotypic frequencies of homozygotes and heterozygotes variants in our study were 73.5% and 26.5%, respectively. These frequencies differ slightly from other frequencies reported in women with vulvovaginal candidiasis. For instance, frequencies of homozygotes and heterozygotes variants in women in China were 66.6% and 33.3%, respectively (20); whereas, these frequencies in women in Brazil were 64.3% and 35.7%, respectively (9). No mutated homozygotes were found in our study nor in the Chinese and Brazilian studies. Very little is known about the association of MBL2 with parasitic infections (12, 27). In the present study, we examined the association of codons 54 and 57 polymorphisms in women with trichomoniasis; however, this infection was present in only 8 individuals and no association of this infection with the polymorphisms was found. The fact that allele B (T) was not associated with any case of cervicovaginal infections suggests that multiple factors other than local factors can be involved in the pathogenesis of vaginal infections.

No statistically significant difference in the frequencies of the codon 54 variants C/C, C/T, and T/T between women with CVI and healthy women was found (68%, 31%, and 1%; and 68%, 29%, and 3%, respectively). Types of CVI were not associated with codon 54 polymorphisms.

With respect to codon 57 polymorphisms, we did not find mutated homozygotes, only seven women with CVI were heterozygotes: two with vulvovaginal candidiasis and five with bacterial vaginosis. Of the healthy women, 13 were heterozygotes, and there was not association between this polymorphism and CVI. This finding is consistent with others reported in the literature (9).

In the present study, all four possible haplotypes were found. However, no association between these haplotypes and CVI was found. Linkage disequilibrium between codon 54 and codon 57 was detected (D'=0.9903, *P*=0.04). To the best of our knowledge, there are no reports in the literature about the link of these haplotypes with acute CVI. MBL2 polymorphisms have been associated with susceptibility to tubal factor infertility (28).

One limitation of the present study was that we did not measure the concentrations of MBL2 protein in vagina. Further studies to determine the association of MBL2 protein concentrations and codons 54 and 57 polymorphisms in Mexican women are needed.

We conclude that MBL2 codons 54 and 57 gene polymorphisms do not associate with CVI in Mexican women.

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ABBREVIATIONS

CI	Confidence interval
CVI	Cervicovaginal infections
MBL	Mannose-binding lectin
OR	Odds ratio

CONFLICT OF INTEREST

The authors declare that no conflicting interests exist.

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