ABSTRACT

Spontaneous abortion occurs in one of every five pregnancies. Infection with Toxoplasma gondii (T. gondii) parasites among many other reasons is accused as a cause of miscarriage. However, it is not subject to unanimity. The current observational cross sectional study aims to shed more light on the potential role of T. gondii infection and the accompanying placental inflammation and Fas ligand (FasL) as a cause of spontaneous abortion in pregnant women from Benha City, Egypt. Sera and placental tissues from 37 women with spontaneous abortion and 25 women with normal delivery were tested for IgG and IgM anti-Toxoplasma antibodies, placental inflammation and FasL expression by ELISA method, histopathological examination of Hematoxylin-eosin staining and immunohistochemical examination, respectively.

Out of 37 abortion cases investigated, 27/37 (73%) were found to be anti-T. gondii seropositive. While only 7/25 (28%) were found in the full term control group. All the T. gondii seropositive abortion cases showed placental villitis and intervillositis. While only two (29%) of T. gondii seropositive control cases show such inflammation. This significant difference was noticed only in anti-toxoplasma IgG seropositive cases but not in IgM positive. FasL expression was detected in all placental tissues of T. gondii positive aborted cases regardless the detected immunoglobulin type. While no FasL expression could be found in placenta of full term control group, even among the toxoplasmosis positive cases. Our study emphasizes the role of chronic toxoplasmosis as a cause of spontaneous abortion and chronic inflammatory lesion and apoptosis mediated by Fas/FasL reaction as possible mechanisms of abortion.

INTRODUCTION

Miscarriage is the spontaneous loss of a pregnancy during the first 24 weeks of gestation. The global incidence of abortion is estimated to be one in five pregnancies. The etiology of miscarriage is not easy to define. Infection among many other reasons is accused as a cause of miscarriage (Giakoumelou et al 2016). About 15% of early miscarriages (less than 12 weeks gestation) and 66% of late
miscarriages (12-24 weeks gestation) have been attributed to infections (Srinivas et al 2006; Baud et al 2008). The infection of mothers with T. gondii parasite during pregnancy can lead to adverse pregnancy outcomes such as miscarriage, stillbirth, and preterm labor or congenital toxoplasmosis (Flegr et al 2014). About 30–50% of the world human population is estimated to be infected with T. gondii ( Robert-Gangneux and Darde 2012) with estimated overall risk of maternal-fetal transmission of the parasite at approximately 30% ( Alvarado-Esquivel et al 2009). Many studies reported an association between toxoplasmosis and spontaneous abortion (Sahwi et al 1995, Gay-Andrieu et al 2003, Anowakowska et al 2006, Amin et al 2012, Galvan-Ramirez et al 2012, Ghasemi et al 2016). However, it is not subject to unanimity and many researchers deny such relation (Stojanović et al 1998, Razzak et al 2005, Qublan et al 2013). In Egypt, although the prevalence of T. gondii infection among Egyptian women ranges between 20% to 67.5%, the relation of toxoplasmosis with high-risk pregnancy was not clearly documented (El Deeb et al 2012, Saleh et al 2014). Understanding the pathogenesis of toxoplasmosis and how it trigger the abortion process is one important step in preventing such adverse pregnancy outcomes. For normal pregnancy to be established, a Th2 type immune response must be induced by the maternal immune system at the maternal-fetal interface (Parslow et al 2001). In pregnant woman infected with T. gondii, this response has two important consequences. First, pregnancy will favor the survival of T. gondii parasite that requires a type 1 response to control them. Second, the established active infection induces a strong shift of immune response from Th2 to Th1 response that adversely could affect pregnancy (Roberts et al 2001). This shift of the immune response during pregnancy may result in induction of placental inflammatory lesions, apoptosis and necrosis of trophoblasts, which can be observed clinically as an abortion process (Benirschke and Kaufmann 2000, Campbell and Lees 2000, Zhao et al 2013). Apoptosis is essential physiological mechanism that is responsible for maternal immune tolerance and allow normal development of the human embryo (Kayisli et al 2003). In toxoplasmosis, studies have suggested that apoptosis starts by extrinsic receptor pathway via overexpression of Fas/Fas ligand (FasL) and play an important role in the pathogenesis of toxoplasmosis (Hu et al 1999, Kitamura et al 2001). The current observational cross sectional study aims to shed more light on the potential role of T. gondii infection and the accompanying placental inflammation and apoptosis as a cause of spontaneous abortion in pregnant women from Benha City, Egypt.

MATERIAL AND METHODS

Study population

The study was carried out on 62 pregnant women aging from 16 to 44 years old attending the Obstetrics and Gynecology Department of Benha University Hospital at the period from February-2014 to December-2014. The women were classified into two groups. The first includes 37 women complaining of spontaneous abortion and emphasized to be free of other common causes of complicated pregnancy (Rh incompatibility, Cytomegalovirus infection, Treponema pallidum infection, teratogenic medica-
tions, positive consanguinity, history of herido-familial problems and history of diabetes mellitus) and have no history of repeated complicated pregnancy. The second group includes 25 women with normal full term gestation enrolled as control group.

The study was approved by the local Ethics Committee and all participant gave consent to do participate in the study.

Samples collection

Two samples were taken from every enrolled woman. One is a serum sample for detection of anti-toxoplasma IgG and IgM antibodies using ELISA technique. Another sample was collected from the placenta for histological examination and immunohistochemistry study for detection of inflammatory lesion and apoptosis, respectively.

Anti-toxoplasma IgM and IgG Enzyme Linked Immunosorbent Assay Tests

Serum samples were tested for the presence of both anti-toxoplasma IgM and IgG antibodies using commercial ELISA kit (Chemux Bioscience, USA) following the manufacturer’s instructions.

Histopathology examination using hematoxylin–eosin stain

Tissue specimens were fixed in neutral-buffered, 10% formalin, routinely processed in paraffin wax, sectioned (4 μm thick) and stained using hematoxylin–eosin for morphological assessment by light microscopy (Gay-Andrieu et al 2003) or examined latter using IHC staining (Wang et al 2012).

Immunohistochemistry detection of apoptosis using FasL detection kit

The paraffin sections were examined using Fas ligand detection immunohistochemistry kit (Thermo Fisher Scientific, Inc., Rockville, MD, USA). Briefly, after deparaffinization in xylene over night, sections were rehydrated and pre-treated in 0.01 M citrate buffer solution (pH 6.0) for antigen retrieval prior to antibody and incubated with 0.3% H2O2 to block endogenous peroxidase activity. For the detection of FasL, sections were incubated with 50 μl of rabbit anti-human FasL antibody (Catalog # RB-9029-R7) diluted at 1:100 overnight at 4°C after tissue sections led in buffer containing normal bovine serum albumin for 20 min at room temperature. Then, sections were incubated with biotin conjugated Goat anti-rabbit IgG (Catalog # 81-6140) for 30 min at 37°C and stained with streptavidin–HRP for 30 min at 37°C. Sections were then incubated in DAB reagent, counterstained with hematoxylin; cover slipped using Canada balsam mounting media. Negative controls were sections incubated with normal goat serum solution without primary antibody (Wang et al 2012). Scoring of the entire section was carried out by using a binocular light microscope at x400 magnification. Positive FasL section was identified when the cell membrane alone or together with the cytoplasm showed brown staining, whereas, negativity was considered when no membrane staining was noticed.

Statistical analysis

The obtained data were tabulated and analyzed using SPSS version 16 software (SPSS Inc., Chicago, ILL Company). Data presented as numbers and percentages. Chi square test (X²) or Fisher's
exact test used as a test of significance. Two sided P <0.05 was considered significant.

RESULTS

Out of 37 abortion cases investigated, 27 (73%) were found to be T. gondii seropositive. A statistically significant lower prevalence (28%) was found in the full term control group. This significant difference was noticed only in anti-toxoplasma IgG seropositive cases but not in IgM positive (table 1).

Histopathological examination of the placental tissue fail to detect T. gondii parasite in any of the studied samples. Our results revealed that 73% of abortion cases show inflammatory criteria as revealed by presence of villitis and intervillitis characterized by chronic inflammatory cells, neutrophils, lymphocytes, histocytes, and few plasma cells (table 1, Fig 1). While only two (8%) of the control full term group showed such lesions (P<0.0001). All the T. gondii seropositive abortion cases showed placental villitis and intervillitis. While only two (29%) of T. gondii seropositive control cases showed such inflammatory lesion. This significant difference (p<0.001) was only found in IgG positive abortion cases when compared with matching control group (table 2).

FasL expression was detected in all placental tissues of T. gondii positive aborted cases regardless the detected immunoglobulin type. While no FasL expression could be found in placenta of full term control group, even among the toxoplasmosis positive cases (Table 2; Fig 2).

Table (1): Frequency of T. gondii infection, villitis and FasL expression in aborted women vs control full term gestation women as revealed by anti-toxoplasma gondii immunoglobulin ELISA, histopathological examination using H&E stain and immunohistochemistry detection of FasL expression, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Total no. examined</th>
<th>Anti-toxoplasma Ig ELISA results</th>
<th>villitis</th>
<th>FasL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG positive No. (%)</td>
<td>IgM positive No. (%)</td>
<td>Ig seropositive No. (%)</td>
</tr>
<tr>
<td>Spontaneous abortion group</td>
<td>37</td>
<td>22 (59.5%)</td>
<td>7 (18.9%)</td>
<td>27 (73%)</td>
</tr>
<tr>
<td>Full term control group</td>
<td>25</td>
<td>7 (28%)</td>
<td>1 (4%)</td>
<td>7 (28%)</td>
</tr>
</tbody>
</table>

* p>0.05= highly significant difference between abortion and full term control groups.  † p>0.001= highly significant difference between abortion and full term control groups.
Table (2): The association between abortion and either FasL expression or villitis in *T. gondii* seropositive aborted women vs full term control using immunohistochemistry (for FasL expression) and histological (for villitis) examination of placentas tissues.

<table>
<thead>
<tr>
<th></th>
<th>T. gondii seropositive cases</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Fas L expression</td>
</tr>
<tr>
<td></td>
<td>IgG positive No. (%)</td>
</tr>
<tr>
<td></td>
<td>IgM positive No. (%)</td>
</tr>
<tr>
<td>Total no. examined</td>
<td>22</td>
</tr>
<tr>
<td>Spontaneous abortion group</td>
<td>22/22 (100%)</td>
</tr>
<tr>
<td>Full term control group</td>
<td>0/7 (0.0%)</td>
</tr>
</tbody>
</table>

*P > 0.001 = highly significant difference between abortion group and full term control.
Fig. (1): Sections of placental tissues from *T. gondii* seropositive patient, stained with H&E. a) Decidua showing lymphocytic infiltration in the stroma. b) Villi showing neutrophil and plasma cells. (x400)

Fig (2): Immunohistochemical detection of FasL in placental villi and decidua from *T. gondii* seropositive women. Rabbit anti-human FasL antibody, biotin conjugated Goat anti-rabbit IgG and streptavidin conjugated to horseradish peroxidase were used as a primary antibody, secondary antibody and detection system, respectively. FasL expression is evidenced by brownish discolor of a) trophoblastic cells of villi and b) decidual cells (x 400).
DISCUSSION

The present observational cross section study revealed association between abortion and infection with *T. gondii* among suspected aborted women when compared with matching full term control group. Our result coincides with previous studies that suggested maternal *T. gondii* infection as a possible cause of abortion (Sahwi et al 1995, Gay-Andrieu et al 2003, Anowakowska et al 2006, Amin et al 2012, Galvan-Ramirez et al 2012, Ghasemi et al 2016). However, the debate about the role of toxoplasmosis in abortion continues. Controversially, some studies reported absence of such relation (Stojanović et al 1998, Razzak et al 2005, Qublan et al 2013). This conflict between researchers could be attributed to unescapable differences between the studied patient groups. In the current work and earlier mentioned supporting studies, the association between abortion and *T. gondii* infection was approved among anti-toxoplasma IgG seropositive patient regardless IgM result. While, IgM positive cases showed no statistical difference when compared with control full term gestation group. Similar conclusion was reported by Sahwi et al who mentioned that there is no relation between abortion and acute toxoplasmosis but present in chronic infection (Sahwi et al 1995). More or less similar conclusion was stated by Chintapalli and Padmaja (Chintapalli and Padmaja 2013) who reported that it is essential to test for both IgG and IgM antibodies, as chronic infection can also lead to fetal loss, as *T. gondii* may encyst in the uterine endometrium and is stirred into activity by the process of placentation. In contrast, Saki et al reported that no significant difference between the abortion cases and control groups in IgG anti-Toxoplasma antibody (Saki et al 2015).

Understanding the pathogenesis of toxoplasmosis is one important step in preventing potential serious pathological effects. The trigger mechanism of abortion in *T. gondii* infected mother is not completely clarified and still needs further studies. Establishment of normal pregnancy requires a complex process involving multiple cell types regulated by several sophisticated mechanisms including implantation, placentation, blood vessel transformation (Michel et al 1990, Ball et al 2006) and state of maternal immune tolerance to the conception (Taglauer et al 2010). An active infection could interfere with the pregnancy by affecting any of these processes leading to miscarriage (Giakoumelou et al 2016). Disruption of materno-fetal interface, as a consequence of inflammatory lesions (Razzak et al 2005) or trophoblast apoptosis (Qublan et al 2013), is suggested as possible pathogenic mechanisms of spontaneous abortion as a sequel to infection. In the current study, histopathological examination of placental samples revealed occurrence of inflammatory changes in the feto-maternal interface in all toxoplasmosis positive abortion cases characterized by presence of villitis and intervillositis. While only 8% of females from normal pregnancy control group have these inflammatory changes. The difference between the two groups is statistically significant (*p* >0.05). Although villitis and the inflammatory infiltrates are not pathognomonic to toxoplasmosis and may be attributed to many other causes (Brito et al 2005), but in our study the complete absence of villitis and intervillositis in toxoplasmosis seronegative abortion
cases favor the presence of association between villitis and toxoplasmosis and potential rule of it as a possible mechanism of abortion. Our finding coincide with that recorded by other researchers who reported that villitis due to toxoplasmosis is characterized by chronic inflammatory cells infiltration and may lead to miscarriage or congenital anomalies (Knox et al 1984, Mederle et al 2008).

The process of programmed cell death, or apoptosis is essential physiological process for immune tolerance to the pregnancy that allow normal development of human embryo (Kawamura et al 2001, Kayisli et al 2003). The Fas/Fas ligand (FasL) system is a major regulator in the induction of apoptosis (Kawamura et al 1999). It provides an efficient mechanism responsible for killing activated lymphocytes and establishes immune privilege in the placenta (Van Parijs and Abbas 1998) and increase apoptosis among glandular and stromal cells during the implantation window allowing the trophoblastic invasion into the endometrium (Kayisli et al 2003, Harada et al 2004). Abnormality of apoptosis during pregnancy can result in miscarriages (Savion et al 2002). The current study detected that, FasL expression is strongly linked with spontaneous abortion in *T. gondii* positive abortion cases when compared with either toxoplasmosis free abortion cases or normal pregnancy mothers. This result comes in agreement with conclusion of Wang et al (2012), Choi et al (2003) and Kaponis et al (2008) who reported significant increase in FasL expression in decidual stromal cells and glandular epithelial cells from aborted women than those from healthy controls. Consequently, it is proposed that apoptosis starting by extrinsic receptor pathway via overexpression of Fas/Fas ligand is one of primary reasons to be involved in pregnancy loss. This finding is supported by the results reported by Hu et al and Kitamura et al who suggested that Fas/FasL interaction play an important role as a trigger for apoptosis in pathogenesis of ocular and pulmonary toxoplasmosis (Hu et al 1999, Kitamura et al 2001).

In conclusion, our study emphasizes the role of chronic toxoplasmosis as a cause of spontaneous abortion and chronic inflammatory lesion and apoptosis mediated by Fas/FasL reaction as possible mechanisms of abortion. Also, our result points the need for researches that focus on interference with the inflammatory changes and apoptosis that may be an important step in controlling the bad obstetric sequel of toxoplasmosis and avoid fetal loss.

REFERENCES


دور داء المقوسات (تكسوبلازم) وما يصاحبه من التهابات مقيمة، وإفراز لبرونق
فاس" كأحد أسباب الإجهاض التلقائي في السيدات الحوامل بمدينة بنيا في مصر
هبه الحسين، وليد العوامي، أمينة عبد المعبد، عزة سعد الغريب،
حسن أحمد حمدتو
قسم الطبيبات - كلية طب بنيا - جامعة بنيا - مصر

يحدث الإجهاض التلقائي في حالة من كل خمس حالات حمل. وتعتبر الإصابة
بطبول التكسوبلازم، جدوى، ضمن العديد من الأسباب الأخرى، سبباً للإجهاض
tلقائي، لكن هذا الرأي ليس محالاً للإجماع. ويهدف هذا البحث الاستقصائي عن طريق
اللاحظة لإظهار مدى من الضوء على الدور المحتمل لعدوى التكسوبلازم وما
يصاحبها من التهابات مقيمة، وإفراز "رابط الناس" كسبب تحدث الإجهاض التلقائي
في السيدات الحوامل، بمدينة بنيا، مصر. تم جمع عينات سبوعية وأجزاء من نسيج
المشيمة من 72 سيدة تعرضت للإجهاض التلقائي. وكذلك من 25 سيدة ودون ولادة
طبيعية. وقد تم عمل اختبار سيرولوجي للكشف عن الأجسام المضادة لطبول
التكسوبلازم من نوعي "م" و "ج". وكذلك تم إجراء فحص نسيجي لعينات المشيمة
لكشف حدوث التهابات مقيمة بالإضافة إلى فحص مناعي-نسيجي لذات عينات
المشيمة للكشف عن إفراز "رابط الناس". وقد أظهرت نتائج الدراسة أن 27 (32%)
من عينات حالات الإجهاض تحتوي على أجسام مضادة لطبول التكسوبلازم، بينما فقط
28% من حالات الولادة الطبيعية كانت موجبة لاختبار الأجسام المضادة، وأظهر
الفحص النسيجي وجود التهابات مقيمة في الرغبات وفي الأجسام المضادة بينما وجدت حالات قطع
(50%) موجبة لاختبار الأجسام المضادة بينما وجدت حالات موجبة
(29%) ضمن عينات المأخوذة من حالات الولادة الطبيعية الموجبة. وأظهر التحليل
الإحصائي أن هذا الفرق يوجد بشكل إيجابي بين الحالات الموجبة لاختبار الأجسام
المضادة ونوع "ج" بينما هو غير معترف بين الحالات الموجبة لاختبار الأجسام
Toxoplasmosis


DARFT

DARFT