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Laminin Is a Promising Predictive Biomarker for Acute and Chronic Toxoplasmosis

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ABSTRACT

Background: Laminin (LN) is an important extracellular matrix glycoprotein plays an important role in early embryonic development by promoting the cell adhesion and angiogenesis. It regulates many functions in the cell including proliferation, invasion, and signaling. However; the role of laminin is poorly known in relation with *Toxoplasma gondii* infection.

Objective: The current study aimed to examine the possibility of using the laminin as an indicator of an early infection of toxoplasmosis.

Methods: Eighty-seven women aged from 15-45 years. They were included in this study from September 2021 to February 2022. Blood samples were collected from healthy pregnant women and aborted and pregnant women infected with toxoplasmosis. Toxo-latex agglutination test was done followed by the detection of IgM and IgG antibodies, which were determined in sera from cases and controls using a commercially available enzyme-linked immunoassay. Finally, an ELISA test for laminin was performed as well.

Results: The seroprevalence of anti-*T. gondii* antibodies IgM or IgG were 14.94% (13/87) and 62.07% (54/87) respectively. Levels of laminin showed a significant decrease in the serum of 1-5 months aborted women with acute and chronic *Toxoplasma Gondii* infection in comparison with the control.

Conclusion: The levels of laminin in female patients infected with *Toxoplasma* G*ondii* might play an important role in early diagnosis of toxoplasmosis especially during the first 5 months of abortion or pregnancy because laminin is an important glycoprotein in the extracellular matrix component, which involved in embryogenesis, implantation, and placentation.

Keywords: *Toxoplasma gondii,* Laminin, ELISA, Toxoplasmosis.

INTRODUCTION

Toxoplasma gondii, is an obligate intracellular parasite, could infect humans and warm-blooded animals, including mammals and birds. It was firstly described in a rodent from North Africa by Nicolle and Manceaux in 1908 and progressively recognized as the agent of a widespread zoonosis [(1)].

The infection with this parasite is known to affect about 30% of the worldwide population, making it as one of the most efficient parasites [(2)]. The infection in humans could be asymptomatic, however it may result in serious complications affecting the vital organs in human body in particular the brain, eyes and fetuses of pregnant women [(2)].

The infection of this parasite has two stages: an acute stage and chronic stage. Early infection of *T. gondii* and the appearance of clinical symptoms coincide with the acute phase. At this stage, the parasite rapidly replicates, and many different cells will be attacked later throughout the body. The patient then enters the chronic phase few months later, where a very slow replication for the parasite would happen [(3)].

The proliferation of the parasite is controlled by the host immune system activation, and tissue cysts would be formed in the neuro-muscular system of the host [(4)]. There is no treatment to a complete elimination of the parasite once the host is infected [(5)].

The contamination through ingestion is the most common mode of transmission, and animals become vectors that will later infect humans who ingest those contaminated meats. The contact with parasites found in cat feces is another mode of transmission to humans. Another ways of transmission are also through transplanted tissues, blood, laboratory accidents and congenital infection (pregnant woman infected with parasites transmits the parasite to her fetus) [(6)].

One of the most important steps in women's lives is pregnancy, specifically for women who intend to have a baby for the first time, while abortion during pregnancy is a problem that any women might experience, which then they face mental issues and medical expenses because of it. Toxoplasmosis infection is one of the reasons for abortion [(7 and 8)]. The prevalence of *T. gondii* infection in pregnant women is measured and is estimated to be 14-77% [(4)].

Congenital toxoplasmosis that occurs during pregnancy could lead to series of complications including stillbirth, spontaneous abortion, and some degrees of physical and mental deterioration, blindness, deafness and hydrocephalus [(9 and 10)].

Furthermore, the gestational age is associated with both the frequency and intensity of the congenital toxoplasmosis. The first and second trimesters showed highest infection severity, which in turn causes either

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abortion or stillbirth. However, the third trimester of pregnancy showed the highest rate of congenital toxoplasmosis [4 and 10]. Early embryonic death refers to the pregnancy ends before organogenesis. When fetus has matured fully in uterus, but is born dead, this is called a stillbirth [11]. The most frequently causes of abortions in human and domestic animals are infectious agents [12].

In healthy pregnant women, the recognition and adhesion of the trophectoderm to the uterine epithelium is the start of the placentation process, the embryonic trophectoderm produce trophoblast stem cells and modify into different trophoblast lineages [13]. Moreover, normal placentation process associates with cell-to-cell and cell-to-ECM (Extracurricular matrix) interactions. Several proteins such as collagen, fibronectin, laminins (LNs) and others are consisting the ECM, which regulates cell functions including signaling, proliferation, migration and invasion. In addition, they are a supply for a placental cells microenvironment [14]. Laminin (LNs) are ECM molecules that is located primarily in basement membranes, and composed of a family of glycoproteins, and interchange with the receptors of cell surface, which play a vital role in the transition of intracellular signals that manage the behavior of the cell [15 and 16]. Laminin is a 900-kDa extracellular matrix glycoprotein, which is responsible for stimulation of the adhesion of the cell and promotion angiogenesis in early development of the embryo [17 and 18].

It is reported that laminin-1 synthesized at the earliest stage of embryogenesis [19]. Immunohistochemical studies showed that one of the most abundant elements of the basement membrane of decidua basalis, placental blood vessels and chorionic villi is laminin-1 [20 and 21]. Furthermore, anti-laminin antibodies were first discovered in 1989 in the sera of recurrent miscarriages monkeys, and it is mentioned that their sera caused abnormalities in cultured embryos of the rat [22].

Furthermore, studies showed that monkeys with multiple successful pregnancies immunized with murine laminin or laminin-1 peptides, namely, YIGSR or RGD, caused the sera to become embryo-toxic, and spontaneous abortions were noted [23]. This finding suggests that anti-laminin-1 antibodies may suppress the implantation of embryo via the inhibition of the invasion of trophoblastic cell into decidua [24].

In human, the detection of laminin-1 chain happened in trophoblastic basement membrane and in normal first-trimester placenta. It is directly contacted with extravillous trophoblastic cells [25]. Throughout gestation, the levels of laminin elevate and during the third trimester, it reaches a plateau, and this might be explained due to the growth of the placenta, which is an organ rich in basement membranes [26].

As mentioned in previous studies, laminin levels are elevated during normal pregnancy as the placenta grow through the months and these levels could be affected by any trauma, pre-eclampsia, tumors, inflammation, virus, liver diseases and fetal death [26 and 27]. Therefore, the current study aimed to report laminin levels, which were measured in aborted and pregnant women infected with toxoplasmosis, and compared them to normal cases. In addition, studying of the possibility of using the laminin as an indicator of an early infection of toxoplasmosis.

PATIENTS AND METHODS

Samples collection: Eighty-seven women were included in this study. Blood samples were collected at Al-Yarmook teaching hospital and then separated and stored at -10°C until they had been analyzed.

Samples testing:

After samples collection, toxoplasma agglutination test was first done according to the manufacturer's instructions. *Toxoplasma gondii*- latex agglutination test (from Spinreact company-Spain) is based on antigen – antibody reaction where the agglutination is occurring when soluble *T. Gondii* antigen coating with latex particles mixed with samples that have antibodies anti *Toxoplasma* [28]. Secondly, all the positive sera for agglutination test were screened for the presence of IgM and IgG antibodies against *T. gondii* by using mini VIDAS kits (Vitek Immuno Diagnostic Assay System from Biomerieux Company).

The VIDAS TOXO IgM (TXM) assay was used to detect IgM and it is an enzyme-linked fluorescent immunoassay (ELFA) that is done in an automated instrument. While, VIDAS TOXO IgG (TXG) assay was used to detect IgG and the principle of the assay is having two steps enzyme immunoassay sandwich method with final fluorescence detection (ELFA). The instrument controlled all assay steps and assay temperature and after completion the assay, the instrument analyzed the results automatically, generated a test results, and printed a report for each sample.

Finally, human laminin ELISA Kit was used to measure human laminin in the sera. The principal work of ELISA kit is a sandwich enzyme-linked immune-sorbent assay procedure. Anti- laminin antibody was pre-coated onto the well plates. In addition, the biotin conjugated anti-laminin antibody was used for antibodies detection. The O.D. absorbance was read at 450 nm using a micro-plate reader, and then the Laminin concentrations were calculated.

Ethics: The Local Ethical Committee of University of Baghdad / College of Science / Department of Biology (Ref. CSEC/0921/0057), approved this work.

Statistical Analysis

The statistical analysis system-SAS (2012) program was perform to identify the effect of difference factors on study parameters [29].

T-test was used for a significant comparison between means. Moreover, Chi-square test was used to significantly compare between the percentages (0.05 and 0.01) probabilities.

RESULTS

The study included the following groups: The control group which included only healthy pregnancies (n=20 including five healthy pregnant women in their 1-5 months of pregnancy, and 15 healthy pregnant women in their 6-9 months of pregnancy).

The test group included 1-5 months aborted patients infected with toxoplasmosis, and non-aborted infected pregnant women (n=49) which in turn included (10 patients with acute toxoplasmosis, 35 patients with chronic toxoplasmosis and 4 non-aborted pregnant women infected with chronic toxoplasmosis), 6-9 months aborted patients infected with toxoplasmosis, and non-aborted infected pregnant women (n=18) which included (3 patients with acute toxoplasmosis, 4 patients with chronic toxoplasmosis, and 11 non-aborted infected pregnant women) as shown in table (1).

Table (1): the number of samples in each group that were included in this study

Groups	N	Aborted Women Acute Toxoplasmosis (IgM)	Aborted Women Chronic Toxoplasmosis (IgG)	Pregnant Women Chronic Toxoplasmosis (IgG)
1-5 Months	**49**	**10**	**35**	**4**
Healthy 1-5 Months	**5**	**-**	**-**	**-**
6-9 Months	**18**	**3**	**4**	**11**
Healthy 6-9 Months	**15**	**-**	**-**	**-**

The highest percentage of infected women with *T. Gondii* was 41.38% at the age group 15-25 years, followed by the women aged 26-35 years, and 36-45 years with ratio 33.33 % and 2.30 % respectively with significant differences (P≤0.01). Out of 87 seroprevalence women, 54/87 (62.07%) were seropositive for IgG antibodies, and 13/87 (14.94%) were seropositive for IgM antibodies with significant differences (P≤0.01) as shown in table (2).

Table (2): The distribution of toxoplasmosis among the groups according to their age

Age	IgM positive	IgG positive	Total	Control
15-25	8/87 (9.19%)	28/87 (32.18%)	36/87 (41.38%)	15/87 (17.24%)
26-35	4/87 (4.60%)	25/87 (28.74%)	29/87 (33.33%)	5/87 (5.75%)
36-45	1/87 (1.15%)	1/87 (1.15%)	2/87 (2.30%)	0/87 (0.00%)
Total	13/87 (14.94%)	54/87 (62.07%)	67/87 (77.01) %	20/87 (22.99 %)
P-value	0.0001 **	0.0008 **		0.0001 **
** (P≤0.01).				

Table (3) showing that there is a significant difference in the concentrations of the human laminin serum among the subjected groups. The first group, which is the 1-5 months aborted women with acute *Toxoplasma gondii* infection, showed a significant decrease in laminin levels in comparison with the control group and for those with the chronic infection. Followed by the pregnant *Toxoplasma gondii* infected women ,which also showed a reduction in serum laminin levels in comparison with the control group.

Table (3): Comparison among the studied groups regarding the concentrations of human laminin in women.

Groups	Mean ± SE of Laminin (ng/ml)		
	Aborted women Acute toxoplasmosis (IgM)	**Aborted women Chronic toxoplasmosis (IgG)**	**Pregnant women Chronic toxoplasmosis (IgG)**
1-5 Months abortion	6.25 ±0.64	9.01 ±0.48	6.88 ±0.69
Healthy 1-5 Months	11.37 ±1.33	11.37 ±1.33	11.37 ±1.33
T-test (P-value)	2.845 ** (0.0020)	1.736 * (0.0488)	3.84 * (0.028)
6-9 Months abortion	10.23 ±1.52	9.64 ±1.52	11.48 ±067
Healthy 6-9 Months	10.22 ±0.68	10.22 ±0.68	10.22 ±0.68
T-test (P-value)	3.576 NS (0.992)	3.576 NS (0.736)	12.69 NS (0.213)
* (P≤0.05), ** (P≤0.01), NS: Non-Significant.			

DISCUSSION

The result of this study showed that the percentage of IgG was more than IgM in most of infected pregnant women with toxoplasmosis, which indicated that these women were infected with chronic toxoplasmosis.

The function of these immunoglobulins including IgG and IgM are essential, due to their role for prevention of cell invasion, activating complement pathways and the catalytic activity of NK and CD8+ T cells that lead to parasite's destroy, or by phagocytosis [(30)].

Explanation for the results in this study might be that laminin, as other previous studies mentioned, is a very important glycoprotein in the component of the extracellular matrix involved in oocyte maturation, embryogenesis, implantation, and placentation [(31)]. Furthermore, there is evidence explained that laminin-1, formed in early human embryos, is found to increase type IV collagenase expression and this is thought to enhance trophoblast adhesion to maternal matrix in the peri-implantation period [(32)]. Other studies showed that LN-1 significantly associated with the recurrent first-trimester miscarriages in humans [(33)].

In addition to the previous component, **Furtado *et al.*** **[(34)]** found that laminin which is found on the parasite surface can bind easily to laminin receptors on many host cells and it is consistent with the ability of the parasite to enter or invade nearly all nucleated cells in human [(34)]. This may explain the low concentrations of laminin as laminin receptors are occupied by those from the *toxoplasma* parasite.

There is a study that has closely related findings with the current results, it had been done on intestinal barrier after ingestion the parasite in the jejunum of infected rats, using immunohistochemistry to label the laminin allowed that ruptures in the basement membrane of the jejunum to be visualized of which suggests that *T. gondii* caused the ruptures. Furthermore, it quantitatively mentioned that there was a significant decrease in the immuno-staining of the laminin after 6 hours of infection [(35)].

CONCLUSION

As a conclusion, previously mentioned can be also an explanation for the reduction in concentrations of laminin in the first trimester abortion for the infected women in the current study and that the same process explained above might be happened in the placenta after the parasite infection.

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