



Immunological Study of Aborted Women Infected with Toxoplasmosis in Basrah Province

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Background: Toxoplasmosis is regarded as one of the chief sources of miscarriage in pregnant women which caused by an apicomplexan obligatory intracellular parasites called *Toxoplasma gondii*. it can infect greatest kinds of warm-blooded animals, together with humans. **Aim:** Assessment some immunological parameter concentration and its relation with aborted women infected with toxoplasmosis by using ELISA method. **Methods:** A total number (155) of serum were collected from women aged between (16-42) years aborted women (75 seropositive with toxoplasmosis, 40 sera negative) were admitted in Basrah hospital for women and children and (40) healthy control women. **Results:** The results show the concentrations of protein S, C, anti-phospholipid and anticardiolipin (68.11, 63.17, 48.59 and 13.86) respectively with significantly than others group. The rate of strong immune factor such as anticardiolipin, protein-C, and protein-S in frequently aborted females is shown to be related to the changes. **Conclusion:** Active *Toxoplasma gondii* infection is more associated with miscarriage than immune factor in this group of experienced females.

Key words: Toxoplasmosis, aborted women, phospholipid, protein S, cardiolipin

INTRODUCTION

Toxoplasmosis is regarded as one of the chief sources of miscarriage in pregnant women which is caused by apicomplexan obligatory intracellular parasites called *Toxoplasma gondii*. It can infect the greatest kinds of warm-blooded animals, together with humans, about 200,000 cases of congenital toxoplasmosis happen in a year.¹ Owing to the lack of overt signs, hosts are susceptible to *T. gondii* infections and may not even be aware that they have toxoplasmosis of the greatest immunocompetent individuals.² Although infection rates vary greatly between nations, reviews of serological investigations have suggested that 30%–50% of the world's population has been exposed to latent toxoplasmosis and may be chronically infected.³ Worldwide, an estimated 73 million induced abortions are carried done annually. Six out of 10 (61%) of all unexpected pregnancies and three out of 10 (29%) of all pregnancies end in an induced abortion.⁴ There are two types of immune responses: adaptive, which develops immune memory after an initial response to

a specific pathogen, and innate, which acts as the body's first line of defense against pathogens but lacks immunological memory when exposed to the same pathogen again.⁵

The test of anticardiolipin is prescribed when a person has symptoms of an autoimmune disorder, such as blood clots or recurrent miscarriages. The Antiphospholipid (APL) test is typically done when a person has a history of blood clots or pregnancy complications. It is used to help diagnose antiphospholipid syndrome (APS), a condition, in which the body produces these antibodies, and the risk of blood clots, strokes, and other health problems is increased. Nowadays, most clinicians would concur that the existence of phospholipid-binding antibodies appears to be linked to a condition characterized by recurrent arterial or venous thrombosis, thrombocytopenia, and recurrent pregnancy losses.⁶ Protein S and protein C are produced in the liver, and their production depends on Vitamin K. They help regulate

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blood clot formation and reduce clotting factors. If there is not a sufficient amount of protein C or protein S, or they do not work properly, this may lead to excessive clotting, activated protein C, and its cofactor protein S work together to break down FVIIIa and FVa on the surface of negatively charged phospholipid membranes, hence preventing coagulation.⁷

The aim of this study is to assess some immunological parameter concentrations and their relation with aborted women infected with toxoplasmosis using the enzyme-linked immunosorbent assay (ELISA) method.

MATERIALS AND METHODS

In this study, an immunodiagnostic kit of German origin was used [Table 1]. Serum samples were collected from a total of 155 women consisting of (75 seropositive aborted women with toxoplasmosis, 40 seronegative aborted women for toxoplasmosis, and 40 healthy control women) in this study, all the aborted women were admitted to Basrah Hospital for Women and Children during the period of November 2023 till February 2024.

The age range of these individuals varied from 16 to 42 years. The research included exclusion criteria for women with several forms of infertility, including tubal obstruction, male infertility, endocrine disorders such as thyroid condition, adrenal disorder, diabetes mellitus, and pituitary disorder. A volume of three milliliters of venous blood was obtained

using vacuum tubes containing gel/clot activator. The collected blood samples were then allowed to stand at room temperature for a duration of 1 h to facilitate the development of clots. Following centrifugation at a speed of 2000 rpm for a duration of 10 min, the serum was carefully aspirated and then distributed into sterile tubes. The tubes were then kept at a deep freeze temperature. The repetition of freezing and thawing in the context of serological research immunoassay was avoided. The levels of total antiphospholipid (APA) anticardiolipin antibodies (ACA), protein S, and protein C were evaluated using the competitive inhibition enzyme immunoassay method, specifically the ELISA. SPSS 22, IBM Corporation, New York, United States of America, 2012 analyses were used to investigate the impacts of exposure to metal combinations, namely APA, ACA, protein S, and protein C. SPSS analyses were used to investigate, quantitative variables were determined and described using the mean and standard deviation, and the *t*-test of significance was utilized, with the lowest level of significance defined as when the probability ($P \leq 0.05$). The correlation coefficient was utilized

Table 1: Immumodiagnostic kit that used in study

Parameter	Company	Origin	Website
Anticardiolipin Ab	AESKULISA	Germany	www.AESKU.com
Antiphospholipid Ab	AESKULISA	Germany	www.AESKU.com
Protein C	AESKULISA	Germany	www.AESKU.com
Portein S	AESKULISA	Germany	www.AESKU.com

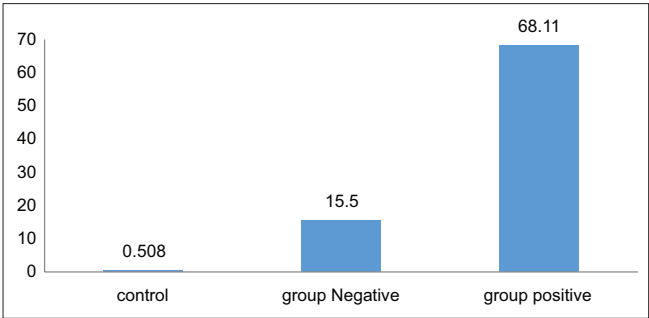


Figure 1: Concentration of protein S level among the study groups

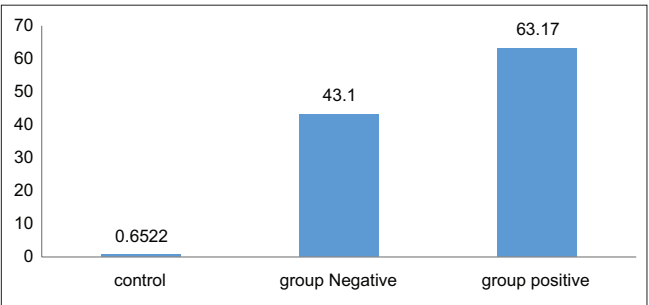


Figure 2: Concentration of protein C level among the study groups

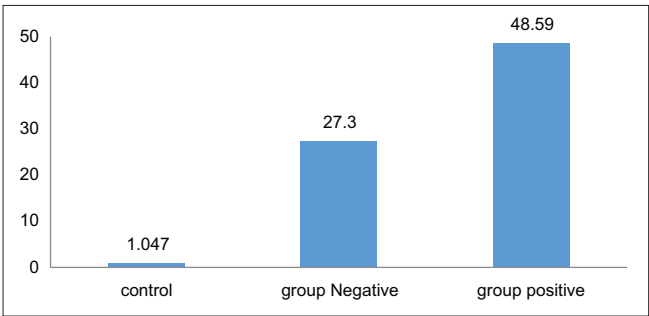


Figure 3: Concentration of antiphospholipid level among the study groups

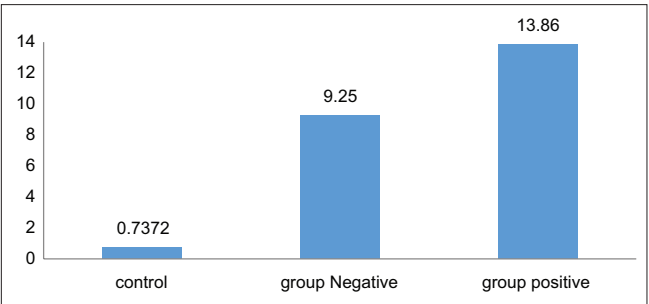


Figure 4: Concentration of anticardiolipin level among the study groups

to determine the strength of the relationship between the variables.

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional review board of the General Directorate of Basra Governorate Health with approval number: 6732 and approved date: November 3, 2023. Patient approval was obtained based on IRB approval.

RESULTS

The concentrations of protein S were detected from the serum of women and controlled by ELISA. Among the two group's aborted women (seropositive and seranegatives infected by toxoplasmosis) and compared to the control group, the highest concentration of protein S was found in the seropositive group (68.11 ± 58.733), whereas the lowest percent was found in the control group (0.508 ± 0.316), with high significant difference ($P = 0.001$) [Table 2 and Figure 1].

The concentrations of protein C were detected from the serum of women and controlled by ELISA. Among the two groups of aborted women (seropositive and seranegatives infected by toxoplasmosis) and compared with the control group, the highest concentration of protein C was found in the group (positive and negative) of (63.17 ± 49.246) and (43.10 ± 60.20), respectively, whereas the lowest concentration was found in the control group (0.6522 ± 1.243), with ($P = 0.008$) statistically, this difference was high significant [Table 3 and Figure 2].

The concentrations of APA were detected from the serum of women and controlled by ELISA. Among the two group's women (seropositive and seranegatives infected by toxoplasmosis) that compared with the control group, the highest concentration of APA was found in the group (seropositive and seronegative) of 48.59 ± 29.401 and 27.3 ± 20.799 , respectively, whereas the lowest concentration was found in the control group (1.047 ± 1.670), with $P = 0.008$ statistically, this difference was high significant [Table 4 and Figure 3].

The concentrations of anticardiolipin were detected in the serum of women and control by ELISA. Among the two group's women (seropositive and seranegatives infected by toxoplasmosis) and compared with the control group, the highest concentration of anticardiolipin was found in the group (seropositive and seranegative) of 13.86 ± 5.328 and 9.25 ± 3.321 , respectively, whereas the lowest concentration was found in the control group (0.7372 ± 0.054), with significant difference ($P = 0.03$) [Table 5 and Figure 4].

Correlations

The interpreting study of correlation observed that there is a very strong positive correlation between immunological protein S with level of immunological parameter including protein C and anticardiolipin respectively. Current study also observed there was a negative correlation between abortion time and level of immunological study protein S, protein C, ant phospholipid and anticardiolipin statistical difference strong negative non-significant ($P = 0.875, 0.551, 0.289$ and 0.049) respectively are arranged in Table 6. Documented data revealed that there were a strong negative correlation of protein S and protein C with level of anti-phospholipid ($P = 0.504, 0.583$) respectively statistically non-significant.

DISCUSSION

Miscarriage has been increasingly associated with toxoplasmosis infection which is considered the most common abortion cause. Furthermore, congenital toxoplasmosis can result in serious prenatal abnormalities such as stillbirth and abortion.⁸ Hence, the community has been evaluated the miscarriages that should be done for toxoplasmosis examined.

The current study shows that the mean results of seropositivity protein S and protein C were highly valued (68.11 ± 58.733 and

Table 2: Total concentration of protein S according to distribution in the study group

Protein S (U/mL)			P
Positive group	Negative group	Control	
68.11±58.733	15.50±53.492	0.508±0.316	0.001

Table 3: Total concentration of protein C according to distribution in the study group

Protein C (U/mL)			P
Positive group	Negative group	Control	
63.17±49.246	43.10±60.20	0.6522±1.243	0.008

Table 4: Total concentration of antiphospholipid according to distribution in the study group

Antiphospholipid (U/mL)			P
Positive group	Negative group	Control	
48.59±29.401	27.3±20.799	1.047±1.670	0.008

Table 5: Total concentration of anticardiolipin according to distribution in the study group

Anticardiolipin (U/mL)			P
Positive group	Negative group	Control	
13.86±5.328	9.25±3.321	0.7372±0.054	0.03

Table 6: Correlation of abortion time with protein C, anti-phospholipid and anticardiolipin according to Positive group

Parameter	Correlation	Abortion Time	Protein S	Protein C	anti-phospholipid	anticardiolipin
Abortion Time	Pearson Correlation	1	0.019	0.070	-0.124	0.228*
	Sig. (2-tailed)		0.875	0.551	0.289	0.049
	<i>n</i>	75	75	75	75	75
Protein S	Pearson Correlation	0.019	1	0.732**	0.078	-0.424**
	Sig. (2-tailed)	0.875		0.000	0.504	0.000
	<i>n</i>	75	75	75	75	75
Protein C	Pearson Correlation	0.070	0.732**	1	0.064	-0.388**
	Sig. (2-tailed)	0.551	0.000		0.583	0.001
	<i>n</i>	75	75	75	75	75
anti phosphor	Pearson Correlation	-0.124	0.078	0.064	1	-0.009
	Sig. (2-tailed)	0.289	0.504	0.583		0.939
	<i>n</i>	75	75	75	75	75
Anticardio	Pearson Correlation	0.228*	-0.424**	-0.388**	-0.009	1
	Sig. (2-tailed)	0.049	0.000	0.001	0.939	
	<i>n</i>	75	75	75	75	75

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed)

63.17 ± 49.246), respectively, in aborted women infected with toxoplasmosis than their aborted women that noninfected with toxoplasmosis and healthy control group with high significant differences and show the high value of antiphospholipid and anticardiolipin infected aborted women is 48.59 ± 29.401 and 13.86 ± 5.328, respectively, than other groups with high significant differences. This results compatibility with the recent study that found the initial line of defense in innate immunity, macrophages are what effectively eradicate *T. gondii*. The immunoregulatory interleukin-12, which coordinates the synthesis of interferon-gamma from diverse immune cells, is the vehicle by which this activity is carried out.⁹ Similar study observed that cases that tested positive for cytomegalovirus (CMV) had elevated protein C values significantly, in the same way, rising significantly antiphospholipid readings in the cases indicated a positive presence of toxoplasmosis, which suggested that early diagnosis and treatment of protein C deficiency and APS, respectively, can be achieved by detecting the infectious organisms responsible for abortions and toxoplasmosis, to reduce the risk of recurrent abortions and high venous thrombotic events.¹⁰

Found the protein S deficiency as a pathological factor for recurrent miscarriage with uterine abnormalities, Vitamin D3 deficiency (3.5%), psychological factors, and autoimmune abnormalities.¹¹ Hence, parental chromosomal abnormalities, uterine deformities, viral illnesses, endocrine disorders, and autoimmune deficiencies are among the several recognized causes of recurrent miscarriage. Even if a parasite infection does not result in infection of the mother or fetus, it may still cause problems for the pregnancy by interfering with any of

the previously listed processes and by upsetting the immune system's homeostasis.¹²

CONCLUSION

Analysis of the data from this study showed that although a significant proportion of each immune factor mentioned was present within the sample of female patients, none could be identified as a critical factor for recurrent miscarriages. The statistical truth in this study indicates that the largest percentage of all miscarriages occurs in cases of toxoplasma infection rather than in cases, in which the infection is negative and is linked to some immune factors. However, many of them can be explained by the occurrence of infectious diseases or the presence of infectious microorganisms; *T. gondii* appears to be the main causative agent in this regard. The rate of strong immune factors such as anticardiolipin, protein-C, and protein-S in frequently aborted females is shown to be related to the changes. Active *T. gondii* infection is more associated with miscarriage than immune factors in this group of experienced females. We do recommend routine screening tests to be conducted in our local teaching hospitals to identify positive cases if any. These screening tests will improve the follow-up for those positive cases which might reduce the rate of continuous abortion in this context.

Data availability statement

The data that support the findings of this study are available from the corresponding author, Baydaa F. Swadi, upon reasonable request.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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