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Original Article

Electrochemiluminescence Epidemiologic Detection of *Toxoplasma gondii* Infection in Pregnant Women With Direct and Indirect Diagnostic Techniques (ELISA Avidity Plus Biochemical Assay), Tehran, Iran

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Abstract

Background and aims: Toxoplasmosis is a very common disease in the world and has two types, including chronic and acute toxoplasmosis. In the chronic toxoplasmosis, the abnormality of tissue function is negligible, but in acute toxoplasmosis, the function of the reticuloendothelial system is interrupted and the assessment of *Toxoplasma* antibody with tissue enzymes is very essential in this stage. In addition, in some conditions, serum ferritin increases in the acute phase of the infectious disease. In congenital toxoplasmosis, the evaluation of biochemical factors and IgG avidity test is important for detecting the acute congenital toxoplasmosis in pregnant women. Based on the above-mentioned explanations, the present study aimed to evaluate the biochemical factors in patients with acute toxoplasmosis (congenital toxoplasmosis) among the Iranian population using electrochemiluminescence and IgG ELISA avidity.

Methods: The study was based on a comparative abundance study and was conducted from March to June 2017. Material included 980 serum and amniotic fluid samples collected from human blood with a high level of IgG antibody against Toxoplasma gondii in Rajaie Center, Tehran, Iran. The standard and the main tests included the ELISA assay and the measurement of the liver transaminases (i.e., SGOT and SGPT), along with/namely bilirubin and ferritin used to detect IgG antibodies and to evaluate the acute toxoplasmosis, respectively. Finally, the results were analyzed by SPSS software.

Results: The results showed that the level of liver transaminases, namely, serum bilirubin and ferritin increase in some patients with a high level of IgG avidity antibody against *Toxoplasma gondii*. For example, the mean serum levels of SGOT was 108 IU/L in 120 patients with acute toxoplasmosis and the mean serum bilirubin was about 5 mg/dL in 80 patients.

Conclusion: Overall, in acute congenital toxoplasmosis, the evaluation of IgG AVIDITY is regarded as the first step and then the measurement of biochemical factors such as serum transaminases, serum bilirubin, and serum ferritin is important.

Keywords: Toxoplasmosis pregnant women, Electrochemiluminescence, SGOT, SGPT, Bilirubin, Ferritin, IgG avidity.

Introduction

Toxoplasma gondii is an intracellular parasite of many kinds of tissues, including the muscle, liver, and intestinal epithelium. In heavy acute infections, the organism can be freely found in the blood and peritoneal exudates. In addition, it may inhabit in the nucleus of the host cell but usually lives in the cytoplasm. The life cycle of *T. gondii* includes intestinal-epithelial and extra-intestinal stages in domestic cats and other felines while it is only observed in extra-intestinal stages in other hosts such as a human. Seropositivity to *T. gondii* is comparable among liver disease patients and controls. Raw shellfish consumption and domestic cat exposure are found as the risk factors for acquiring acute *T. gondii* diseases. In the United States, exposure to certain raw or undercooked foods and the kittens are considered as the risk factors for *T. gondii* infection.[1-3]

Considering the fact that antibody to *Toxoplasma* is widely prevalent in hosts throughout the world, the clinical toxoplasmosis is yet less common. The general use

of the IgG avidity Toxoplasma test can solve the diagnosis in IgG positive in congenital toxoplasmosis. It is clear that most infections are asymptomatic or mild. In 1976, the global prevalence of toxoplasmosis was estimated at over 500 million. In the acute phase, tachyzoites proliferate in many tissues and this rapid reproduction tends to kill the host cells at a faster rate than does the normal turnover of such cells. As infections become chronic, the zoites infecting the brain, heart, liver, and the skeletal muscles multiply much more slowly compared to the acute phase. *T. gondii* causes massive necrosis of the liver cells and chorioretinitis.[4-7] In various immune suppressed hosts *T. gondii* becomes acute and acute toxoplasmosis may lead to congenital toxoplasmosis, along with fetal complication outcomes during the pregnancy. The *Toxoplasma* infection may lead to adverse pregnancy outcomes; therefore, developing good habits of life and health is an effective way to avoid such pregnancy outcomes.[8-10]

Further, *T. gondii,* is one of the infectious agents of congenital TORCH infections, which causes severe clinical outcomes in fetus and newborns. And infection with *T. gondii* during pregnancy can lead to severe fetal sequelae. Similarly, ocular toxoplasmosis is a disease caused by the infection with *T. gondii* through congenital or acquired routes. Different laboratory methods used for diagnosing the congenital toxoplasmosis have variable sensitivity and specificity such as IgM Western blotting, IgA enzyme immunoassay, and DNA amplification by the real-time polymerase chain reaction. In the acute stage of toxoplasmosis, the level of IgG avidity antibody is high and thus in this condition, the level of the tissue enzymes such as transaminases (i.e., SGOT and SGPT), serum bilirubin and ferritin increases and the total malfunction of the reticuloendothelial system is observed accordingly. The increase in serum transaminases (i.e., bilirubin and ferritin) can be important in the acute phase of intracellular infection.[11-15]

Objective

The purpose of this survey was to investigate biochemical factors using the electrochemiluminescence assay and ELISA avidity test in patients with acute congenital toxoplasmosis (i.e., IgG avidity positive) among the Iranian population.

Materials and Methods

The study was based on comparative research. The study samples were serum and amniotic fluid specimens which were collected from 980 patients with high a level of IgG avidity antibody against *T. gondii* in Rajaie Center, Tehran, Iran. The ELISA assay, as the gold standard test, was utilized to detect IgG avidity *Toxoplasma* antibody. The patients had positive ELISA titer for *Toxoplasma* antibody that was measured by laboratory technicians in Rajaie Clinical Laboratory. Furthermore, the main test for detecting the serum liver transaminase and bilirubin was based on the electrochemiluminescence assay and the measurement of serum ferritin based on ELISA assay.

ELISA Method for Detecting Toxoplasma Antibody or Serum Ferritin

1. 100 λ of blood serum, amniotic fluid sample, or calibrator 2 was added to ELISA avidity wells;
2. The samples were incubated for 30 minutes at 37 °C;
3. The plates were washed three times with phosphate buffered saline (ELISA WASHER);
4. 100λ enzyme conjugate was added to these plates;
5. The mixture was incubated for 30 minutes at 37°C;
6. Then, a 100 λ substrate (TMB) was added to the mixture.

After 15 minutes, the stop solution was added and then read at 450 nm ELISA reader.

The Formula of ELISA Avidity (Semi-quantitative):

$$\frac{\text{Optical density (OD) patients}}{\text{OD calibrator 2}}$$

The normal range is <0.8, but the result >1.1 is considered positive for congenital toxoplasmosis.

Formula of SGPT Measurement

l-alanine + 2-oxoglutarate —SGPT→ l-glutamate + pyruvate

pyruvate + NADH + H^+ —LDH→ l-lactate + NAD^+

The reagent 1 for measuring the SGPT consists of TRIS buffer, l alanine, and LDH.

The reagent 2 for estimating the SGPT contains 2-oxoglutarate and NADH.

Formula of SGOT Measurement

l-aspartate + 2-oxoglutrate —SGOT→ l-glutamate + oxoglutarate

oxoglutarate + NADH + H^+ —MDH→ l-malate + NAD^+

The reagent 1 to measure SGOT consists of TRIS buffer, l-aspartate, malate dehydrogenase (MDH) and LDH.

The reagent 2 to measure SGOT consists of 2-oxoglutarate and NADH.

In both measurements, 100 λ human serum + 1000 λ reagent 1 were mixed and after 5 minutes, 250λ reagent 2 was added, followed by reading the OD of reaction tubes against the blank tube at 340 λ wavelength. The normal range (activity) of SGOT and SGPT in this assay was <31U/L. The serum bilirubin, based on the photometric assay, and serum ferritin were measured by the auto-analyzer cobas e411 electrochemiluminescence instrument and ELISA method, respectively.

This study was based on a comparative abundance study and was performed during March-June 2017 and the

obtained results were calculated and analyzed by the SPSS software, version 19.

Results

Based on the results, the level of liver transaminases, namely, serum bilirubin and ferritin increased in a number of patients with a high level of IgG AVIDITY antibody against *T. gondii*. For instance, in 120 patients with acute toxoplasmosis, the mean serum level of SGOT was found to be 108 IU/L, while in 80 patients the mean serum bilirubin was about 5 mg/dL.

As shown in Figure 1, the results showed that the mean of ELISA avidity titer was high in the serum samples of 980 toxoplasmosis pregnant women compared to amniotic fluid sample and thus the mean of ELISA avidity titer increased in each abortion. For example, the mean of ELISA avidity titer was higher in mothers with fourth abortion compared to those with first abortion.

Based on the data in Table 1, the serum levels of bilirubin with 95% CI of the difference were between 1.1 and 1.7 mg/dL in 980 patients with acute toxoplasmosis, which is considered a normal range.

The results showed that only 12% of the patients had a high level of serum SGOT among 980 patients with acute toxoplasmosis (Figure 2).

It was found that only 14% of the patients had a high level of serum SGPT among 980 patients with acute toxoplasmosis (Figure 3).

The obtained that demonstrated that in 980 patients with acute toxoplasmosis, only 8% had a high level of serum bilirubin (Figure 4).

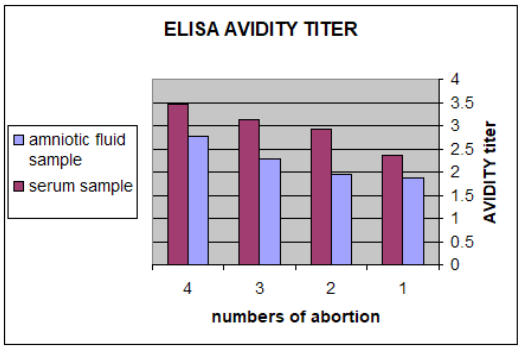

Figure 1. Titer ELISA Avidity From 98 Toxoplasmosis Pregnant Women.

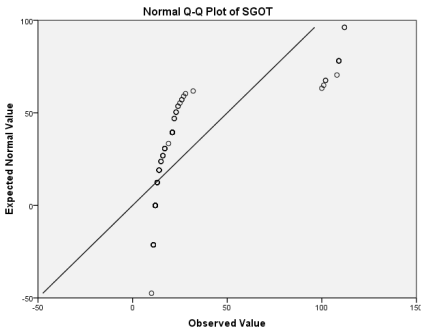

Figure 2. The Q-Q Plot of Serum SGOT in Acute Toxoplasmosis Patients.

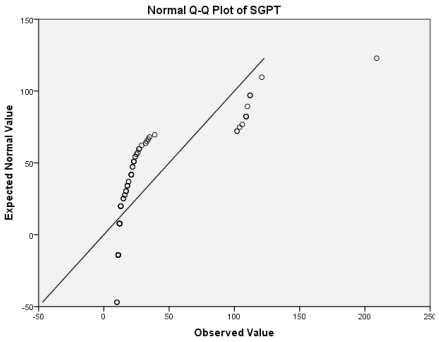

Figure 3. The Q-Q Plot of Serum SGPT in Acute Toxoplasmosis Patients.

Based on the findings, only 14% of the patients had a high level of serum ferritin among 980 patients with acute toxoplasmosis (Figure 5).

According to Table 2, the minimum and maximum level of serum ferritin was 12 mg/dL and 994 mg/dL, respectively, in 980 patients with acute congenital toxoplasmosis. Moreover, the biochemical assay was based on the electrochemiluminescence assay.

Discussion

Toxoplasmosis, as one of the TORCH infections in pregnant women, is caused by *T. gondii*. The seroprevalence of toxoplasmosis among the pregnant women was found to be comparatively high, for example, compared with previous reports from Saudi Arabia or

Table 1. One-Sample *T* Test from Toxoplasmosis Patients

	One-Sample Test					
	Test Value = 0					
	T	*df*	Sig. (2-tailed)	Mean Difference	95% CI of the Difference	
					Lower	Upper
SGOT	9.078	97	0.000	27.64286	21.5992	33.6865
SGPT	8.434	97	0.000	31.30612	23.9392	38.6731
Bilirubin	10.451	97	0.000	1.44388	1.1697	1.7181
Ferritin	5.884	97	0.000	184.34694	122.1611	246.5328

Abbreviations: SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase.

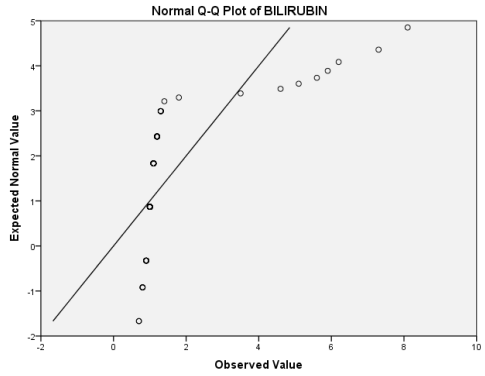

Figure 4. The Q-Q Plot of Serum Bilirubin in Acute Toxoplasmosis Patients.

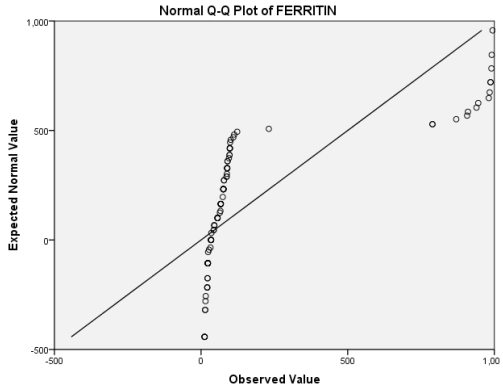

Figure 5. The Q-Q Plot of Serum Ferritin in Acute Toxoplasmosis Patients.

the prevalence of *T*. IgG antibodies in pregnant women was low in Sicilian population. The clinical spectrum of *T. gondii* infection varies from an asymptomatic state to severe illness. Additionally, another study evaluated the preliminary diagnostic utility of the two mixtures of *T. gondii* recombinant antigens in IgG ELISA and IgG avidity test. In addition, an epidemiological study reported an association between *T. gondii* infection and the liver cirrhosis; therefore, high positive results should be treated with some skepticism until additional precise diagnostic tools are developed in this respect. Our purpose was to evaluation biochemical factors using the electrochemiluminescence assay and ELISA avidity test in patients with acute congenital toxoplasmosis (i.e., asymptomatic IgG avidity positive) among an Iranian population.[16-20]

In a recent study in the USA, the elevated risk of recent *T. gondii* infection was associated with eating the rare lamb. Even frozen lamb meat was found to be associated with acute *T. gondii* infection in Brazil. Interestingly, toxoplasmosis with liver involvement was reported in deer hunters who ate the undercooked venison. Under normal immune conditions, *Toxoplasma* infection is largely asymptomatic, but in individuals who are immunocompromised such as those with AIDS, malignant patients under chemotherapy, or organ transplant recipients, the parasite can become widely disseminated, causing severe toxoplasmosis and/or encephalitis. Further, the prevalence of acute *T. gondii* infection is high among children with non-specific reactive hyperplasia of the cervical lymph nodes. The toxoplasmosis is most commonly diagnosed by detecting the immunoglobulin (i.e., IgG and IgG avidity) antibodies in the serum samples of the patients using a variety of methods such as ELISA, IFA, IgM-ISAGA, and the like.[21-25]

The congenital toxoplasmosis is sometimes transmitted by the undercooked meat. A view of the fact that antibody to *Toxoplasma* is extensively prevalent in human throughout the world, the clinical toxoplasmosis remains less common and the occurrence of congenital toxoplasmosis is still a problem. It is clear that most infections are asymptomatic or mild. Several factors influence this phenomenon such as the virulence of the strain of *Toxoplasma*, the susceptibility of the individual host species, the age of the host, and the degree of the acquired immunity of the host. Occasionally, the circumstances conspire to make a mild case important. For example, Elahian et al suggested that there is a significant relationship between the age of the youngest child and the infection rate.[27] Since there seems to be an age resistance, the influence of adults or weaned juveniles is asymptomatic although exceptions occur as well. However, asymptomatic infections can suddenly become fulminating if immunosuppressive drugs such as corticosteroids are employed for other conditions. The mesenteric lymph nodes and the parenchyma of the liver are the first extra-intestinal sites that are infected in both cats and other hosts (humans). Furthermore, these

Table 2. Descriptive Statistics of Biochemical Index in Acute Toxoplasmosis Patients

Descriptive Statistics					
	N	Minimum	Maximum	Mean	SD
SGOT	980	10.00	112.00	27.6429	30.14475
SGPT	980	10.00	209.00	31.3061	36.74527
Bilirubin	980	0.70	8.10	1.4439	1.36775
Ferritin	980	12.00	994.00	184.3469	310.17365
Valid N (listwise)	980				

Abbreviations: SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase.

sites experience rapid regeneration of cells and perform an effective preliminary screening of parasites. The most common symptoms of acute toxoplasmosis are painful swollen lymph glands in the cervical and abnormality in the reticuloendothelial system. In the chronic phase, the cyst of *Toxoplasma* can remain intact for years and produce no obvious clinical effect. The larger number of acute toxoplasmosis (about 70%) is asymptomatic. Mumcuoglu et al suggested to perform *T. gondii* IgM, IgG, and IgG avidity tests by VIDAS automated analyzer using TOXO IgM, TOXO IgG II, and TOXO IgG avidity kits.[28] In this study, we showed that in most asymptomatic acute toxoplasmosis patients with good immune systems, the biochemical factors such as serum SGOT, serum SGPT, serum bilirubin, and serum ferritin had different levels and thus required further study using other methods such as ELISA AVIDITY test. In acute congenital toxoplasmosis, the evaluation of IgG AVIDITY was the first step, followed by measuring biochemical factors such as the serum transaminases, that is, serum bilirubin and serum ferritin.[26-33]

Ethical Approval

This study was approved by the Ethics Committee of Tarbiat Modares University in 2015 (No. 52/3595), provided that it does not lead to distress.

Conflict of Interest Disclosures

None.

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None.

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