Evaluation of Alpha- fetoprotein as a Marker for Toxoplasmosis

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Abstract

Toxoplasmosis is one of the causative agents in women abortion and congenital deformity outcomes. Alpha-fetoprotein (AFP) is a glycoprotein elevated in some carcinoma cases. The obejective of this study was to evaluate AFP as a marker for toxoplasmosis and screening test for a subsequence development of abnormalities. Ninty six blood samples from 15-46 yeas old aborted Iraqi women with Toxoplasmosis were collected and included in this study. The results of investigations were compared with those for 79 apparently healthy volunteers (control group) with no previous abortion. This study reveals that there is a highly significant increment in seropositivity rate of toxoplasma-specific IgM antibodies (67 cases; 69.8%), and Alpha-fetoprotein (72 cases; 75%) in sera of infected aborted women in comparison to healthy controls (P= 0.032). The current results showed that there was highly significant elevation in the levels of AFP (129.23±16.76) as well as *Toxoplasma gondii*-specific IgM antibodies (1.294±0.559 Ng/ mL) in comparison with control group (10.65±5.2 and 0.43±0.2 Ng / mL for AFP and *Toxoplasma gondii*-specific IgM antibodies, respectively) (P=0.001 for both). Moreover, in spite of its highly significant efficiency in comparison with control group (P=0.001), application of ROC test for AFP evaluation showed that the values of the sensitivity, specificity, and accuracy for AFP were 75%, 73.4% and 74.23%, at an optimum concentration of 10 Ng/ mL. Regarding the Toxoplasma-specific IgM antibodies, the sensitivity, specificity, and accuracy values were 100%, 69%, and 83.49%, respectively at optimum concentration of 1 Ng/ mL (P= 0.001). In view of the above results, it could be concluded that α-fetoprotein may be beneficial for screening congenital abnormalities and abortion during toxoplasmosis; while anti-*Toxoplasma gondii* IgM is considered the best and golden standard test for detection of toxoplasmosis.

Key Words: Toxoplasmosis, Alpha- fetoprotein, Abortion, Elisa, Toxoplasma-specific IgM antibodies.

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تقييم بروتين الفا الجنيني كمؤشر على داء المقوسات لدى النساء العراقيات المجهضات

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ا لخلاصة

يعتبر داء المقوسات من أحد المسببات للأجهاض لدى الحوامل والتشوهات الجنينية الناتجة. أن بروتين الفا الجنيني هو بروتين سكري يرتفع في حالات السرطانية . تعد هذه الدراسة كمحاولة لتقييم بروتين الفا الجنيني كمؤشر لداء المقوسات وأختبار تقصٍ عن تكون التشوهات لاحقاً. ضمت هذه الدراسة 96 عينة لنساء عراقيات بداء المقوسات مجهضات تتراوح اعمارهن بين 15-46 سنة. قورنت نتائج التحقيقات تلك بنظائرها لـ 79 عينة إمرأة متطوعة وممن يبدون من الأصحاء ولم يتعرضن للأجهاض (مجموعة سيطرة). أظهرت هذه الدراسة وجود زيادة عالية المعنوية في الـ IgM لدى 67 إمرأة مجهضة (69.8%) وإيجابية لدى 72 (75%) منهن في بروتين الفا الجنيني مقارنة بمجموعة السيطرة (قيمة الأحتمالية =0.032). بينت النتائج الحالية أن هناك إرتفاعا عالي المعنوية في معدل مستوى بروتين الفا الجنيني (129.23 ± 16.76) نانوغرام / مل وكذلك أضداد المقوسة الكوندية نمط anti-*Toxoplasma gondii* IgM (1.294 ±0.559) نانوغرام / مل مقارنة بمجموعة السيطرة 10.65 ± 5.2 و 0.43 ± 0.2 لكل من بروتين الفا الجنيني و أضداد المقوسة الكوندية نمط أم على التوالي (قيمة الأحتمالية = 0.001 لكلٍ منهما. فضلاً عن ذلك وعلى الرغم من الفارق المعنوي للبروتين الفا الجنيني مقارنة بالأصحاء (قيمة الأحتمالية = 0.001) فان تطبيق إختبار ROC test لتقييم بروتين الفا الجنيني أثبت أنه يمتاز بحساسية تبلغ 75% وقيمة مناوعة 73.4% وبدقة تصل قيمتها الى 74.23% وأمثل تركيز يعتدّ به هو 10 نانوغرام / مل. بينما سجلت أضداد الـ IgM مناوعة مطلقة (100%) وحساسية 69.8% ودقة بلغت 83.49% وكذلك بفارق عالي المعنوية مقارنة بالأصحاء في تركيز أمثل يصل الى 1 نانوغرام / مل (قيمة الأحتمالية = 0.001) . في ضوء النتائج أعلاه , يمكن الأستنتاج أن بروتين الفا الجنيني يكون ذا فائدة كإختبار تقصٍ عن حالات التشوهات والأجهاض الناتجة عن داء المقوسات ؛ بينما تبقى أضداد المقوسة الكوندية الأختبار القياسي الذهبي أو الأمثل للتحري عن المقوسات الكونيدية.

الكلمات المفتاحية: داء المقوسات, ألفا فيتو بروتين, الأجهاض, اللايزا.

Introduction

Many causes are standing behind women abortion [1] and congenital toxoplasmosis is one of them [2, 3]. It was reported that Alpha-fetoprotein (AFP) is produced initially by the yolk sac and later by the fetal liver and gastrointestinal (GI) tract of a human fetus, but may also be found at an elevated level in the sera of adults having certain malignancies [4]. It was demonstrated that the fetus excretes AFP into the amniotic fluid and can cross the placenta and enter the maternal circulation, where it can be measured using an immunoassay [5]. Although its real biological role still vague, it is still suspected to be involved in embryonic and fetal development [5-7].

AFP measurements in amniotic fluid are used for the early diagnosis of fetal neural tube defects, such as spina bifida and anencephaly.
Elevated serum levels may be present in ataxia-telangiectasia syndrome, hereditary tyrosinemia, cirrhosis, alcoholic hepatitis, hepatocellular carcinoma, and viral hepatitis. Although, it is not a specific genetic marker for malignancies, AFP may be used to monitor the effectiveness of surgical and chemotherapeutic management of hepatomas and germ cell neoplasms [8,9].

The fact that malformations decrease the integrity of natural barriers between the fetus and the amniotic fluid, hence, increases the chance for transferring of fetal substances into the amniotic fluid with elevated maternal serum AFP levels [10]. In contrast, chromosomal abnormalities are associated with decreased maternal serum AFP levels, for unknown reasons [11]. Assessment of maternal serum levels of AFP is adopted in prenatal testing protocols in many countries [12].

It was proposed that AFP is elevated during pregnancy. Persistence of AFP in the mother following birth is a rare hereditary condition [13]. Neonates have markedly elevated AFP levels (>100,000 ng/mL) that rapidly fall to below 100 ng/mL by 150 days and gradually return to normal during their first year [13,14]. Levels more than twice or less than half the median AFP value for a given gestational week signal the need for additional testing to rule out any abnormalities. Such abnormalities may be attributed to *Toxoplasma gondii* infection.

The objective of the present study was to evaluate AFP as a biomarker for fetal abnormalities development due to infection with *Toxoplasma gondii.*

Materials and Methods

Three hundred and sixty four blood samples from aborted women have been collected during the period from 15th May 2014 to 1st February 2015, who attended Al-Sadir Hospital, Ibn-Albalady Hospital, and Medical City Teaching Hospital.. The age of women ranged from 16- 46 years. The seropositivity rates of anti-*Toxoplasma gondii* IgG and IgM antibodies in addition to α-fetoprotein were compared with 79 blood samples collected from apparently healthy, non-aborted volunteers (control group). Enzyme linked immunosorbent assay (ELISA) was applied for detection of the above parameters [anti-*Toxoplasma* IgM and IgG) in serum (Elisa TOXO IgM and IgG, Biotik, USA) and Alpha-protein serum (Elisa, Monodind Inc. USA) according the to the manufacture instructions].

Statistical Analysis

All the results had been statistically analyzed using t-Test, Chi-square and ROC tests and according to SPSS statistical analysis program version 18 [15].

Results

1. Estimation of anti-*Toxoplasma gondii* IgM and AFP positivity rate in the sera of the studied groups

It can be seen from Table 1 that 69.8% of the aborted women were positive for anti-Toxoplasmosis IgM antibody while 75% of them were positive for AFP with highly significant differences in comparison with the control group (0.0% and 26.6% for IgM anti-*Toxoplasma gondii* IgM antibodies and AFP, respectively) (P= 0.01).

Table 1: Positivity of IgM Anti-*Toxoplasma gondii* & AFP among the sera of the studied groups

Anti-Toxoplasmosis IgM		Studied groups		Total	Chi-Square (P-value)
		Healthy Control	Aborted women		
Negative	N	79	29	108	P=0.00 HS (P<0.01)
	%	100.0%	30.2%	61.7%	
Positive	N	0	67	67	
	%	0%	69.8%	38.3%	
Total	N	79	96	175	
	%	100.0%	100.0%	100.0%	
Alpha fetoprotein (AFP)					
Negative	N	58	24	82	P=0.00 HS (P<0.01)
	%	73.4%	25.0%	46.9%	
Positive	N	21	72	93	
	%	26.6%	75.0%	53.1%	
Total	N	79	96	175	
	%	100.0%	100.0%		

2. Levels of anti-Toxoplasma gondii IgM and AFP in the sera of the studied groups

Estimation of anti-*Toxoplasma gondii* IgM and AFP concentrations in Ng/mL in the sera of the aborted women in comparison with healthy control group was listed in Table 2. It is clear from this table that there was an elevation in the concentrations of AFP (129.23±16.76 Ng/ mL) as well as anti-*Toxoplasma gondii* IgM (1.294±0.559 Ng/ mL) in the sera of the aborted women in comparison with control group (10.65±5.2 & 0.427±0.2 ng / mL for AFP and anti-*Toxoplasma gondii* IgM, respectively) (P=0.001 for both).

Table 2: Concentrations (Ng/mL) of anti-*Toxoplasma gondii* IgM and AFP in the sera of the studied groups.

Parameters	Studied groups	N	Mean (Ng/ mL)	Std. Error	t-test (P-value)
Anti-Toxoplasmosis IgM Antibody	Control group	78	0.43	0.022	P=0.001 HS (P<0.01)
	Aborted women	96	1.294	0.057	
	Total	174			
Alpha fetoprotein (AFP)	Control group	79	10.65	0.585	P=0.00 HS (P<0.01)
	Aborted women	96	129.23	17.103	
	Total	175			

3. Evaluation of anti-Toxoplasma gondii IgM and AFT by ROC test

For evaluation of those two parameters to be applied in routine diagnosis of toxoplasmosis, perhaps as indicators for fetal abnormalities and abortion; ROC test was used and the results are shown in Table 3.

Figure 1 represents the above findings for both AFP and anti-*Toxoplasma gondii* IgM

Table 3: Evaluation of AFP and anti-*Toxoplasma gondii* IgM in order to discriminate between aborted and healthy women.

Validity tests	Alpha fetoprotein	Anti-Toxoplasmosis IgM
Sensitivity	**75%**	**69.8%**
Specificity	**73.4%**	**100 %**
Positive predictive value	**77.419 %**	**100 %**
Negative predictive value	**70.73 %**	**73.148 %**
Accuracy	**74.285 %**	**83.428 %**
Optimum Concentration (Cut-off)	**10.0 ng/ mL**	**1.0 ng/ mL**
Area Under the Curve	**0.759**	**0.849**
P-value	**0.001 HS**	**0.001 HS**

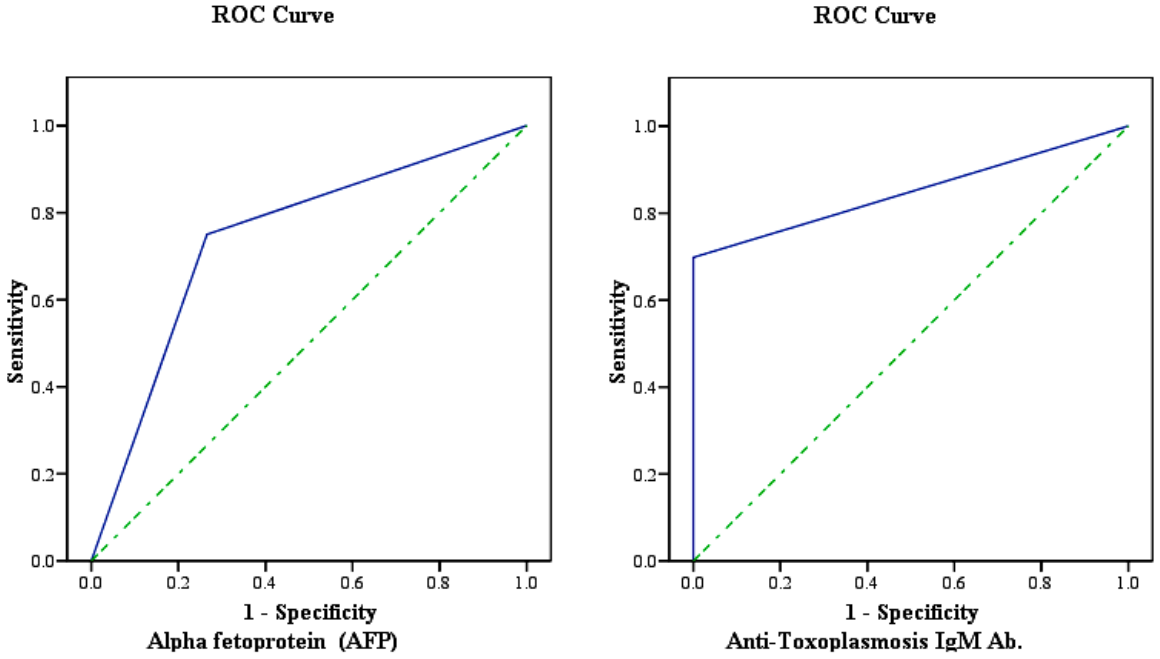

Figure 1: ROC curves for evaluation of AFP and anti-*Toxoplasma gondii* IgM

1. Correlation of AFT and anti-Toxoplasma gondii IgG and IgM

Table 4 showed that there was highly significant correlation between IgM anti-*Toxoplasma gondii* and AFT together with IgG.

Table 4. Correlation between AFP and IgM anti-*Toxoplasma gondii* in addition to other parameters.

Parameters	Pearson Correlation	Anti-Toxoplasmosis IgM	Anti-Toxoplasmosis IgG
Alpha fetoprotein (AFP)	R	0.431	0.843
	P-value	0.0001	0.0001
	Significance	HS*	HS*

*HS means highly significant.

Discussion

Incidence of toxoplasmosis is varied according to the region and the method of detection and thus it varied from 81.8% among Iraqi women in Al-Ramadi city (Using Latex Agglutination Test) to 15.1% among Chinese pregnant women (By application of ELISA technique) [16,17]. In July 1977, AFP was added to the panel of prenatal screening using radioimmunoassay (RIA) technique [18]. The elevation in the level of maternal AFP during the first 15-20 weeks of pregnancy followed by amniocentesis was noticed to be related to natal malformation such as open neural tube malformation [19]. This finding together with others motivated us to conduct this investigation. This study was planned to evaluate AFP as a biomarker which may be dependent anti-*Toxoplasma gondii* IgM for detection of toxoplasmosis in addition to a possibility for application of this test as an indicator for prenatal abnormalities. Many studies denoted to the first biomarker, however no evaluation was carried on previously applied ROC test. Furthermore, no previous studies dealt with the relationship between AFP and other biomarkers. Thus, the diagnosis of toxoplasmosis as early as possible may enhance the treatment and prevention of resulting abnormalities. Most studies focused on anti-*Toxoplasma gondii* IgG for detection of this condition. However, it is better to rely on IgM as soon as possible for the diagnosis of toxoplasmosis [19]. Meanwhile, the detection of abnormalities is still very essential and any screening test for its detection is of importance which in this case perhaps determines the priority for abortion decision or fetal survival. AFP was found mainly to be originated from the fetal yolk sac endoderm and liver beside some from intestinal epithelium of the fetus, according to *AFP* gene coding for it which situated at 4q25 locus on chromosome 4. Any variation in *AFP* related to difference in the gene length [20]. Furthermore, the biochemical studies referred to AFP capacity for binding to copper, nickel, fatty acids and bilirubin [21].

The level of AFP ranges from 200 to 400 ng/ mL during the first trimester while it elevates at 14 weeks of gestation from 25.6 ng/mL up to 74.9 ng/mL at 21 weeks of gestation [22,23].

It was reported that the normal concentration of AFP is less than 5.4 ng/mL (5.4 µg/L) which is in concordance with the results of the present study in which the optimum level was found 10 ng/mL [24,25]. Evaluation of AFP by ROC test revealed highly significant correlation between IgM anti-*Toxoplasma gondii* and AFP although; the specificity, sensitivity and accuracy of the latter are less than the first one. These data attributed to linkage between the antibodies and the parasite, while AFP is well known to be associated with carcinoma cases particularly liver carcinoma and malformation [26]. Furthermore, these findings explain why it is more reliable to depend on AFP as a biomarker for fetal malformation and other abnormalities. Hence, these evidences referred to the importance of anti-*Toxoplasma gondii* IgM in the diagnosis of toxoplasmosis. On the other hand, AFP can be used as a screening test for detection of fetal toxoplasmosis abnormalities [25,26]. For this reason, it is recommended to include this test within the toxoplasmosis diagnostic screening schedule.

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