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AECA and ANA in threatened abortion women in Babylon province

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ABSTRACT— Threatened abortion is a common pregnancy complication that affects approximately 20% of all pregnancies. The aim of this study is to assess the presence of autoantibodies (anti-endothelial cell antibodies and antinuclear antibodies) in the study groups and their significance in the diagnosis of threatened abortion. The study was carried out in the Iraqi city of Babylon. This study included 90 subjects who were separated into three groups: 30 threatened abortion patients, 30 healthy pregnant women, and 30 healthy non pregnant women. All of the study groups were between the ages of 20 and 35 years old. Assessment of autoantibodies (AECA and ANA) in the studied groups blood samples from all subjects were taken. It was made using the Enzyme linked Immunosorbent Assay (ELISA) technology. AECA positive frequency in TA group was (13.3 %) with a significant difference (p= 0.015). While the ANA positive frequency in TA group was (10.0 %) with a significant difference (p= 0.045). In conclusion, immunological tests (AECA and ANA) play an important role in prognosis and/or diagnosis of threatened abortion.

KEYWORDS: AECA, ANA, threatened abortion, pregnancy, autoantibody

1. INTRODUCTION

Threatened abortion is defined as bleeding within the first 20 weeks of gestation when the cervix is closed. In fact, this is the most common pregnancy complication, occurring in approximately 20% of all pregnancies. In around 50% of the cases, the disease might lead to miscarriage. A speculum examination should be performed as part of the first evaluation to rule out cervical or vaginal hemorrhage. A physical assessment is also necessary to rule out extragenital hemorrhage and ectopic pregnancy [1].

Anti-endothelial cell antibodies (AECA) and antinuclear antibodies (ANA). These autoantibodies have been linked to immunological failure during pregnancy, which leads to miscarriage. AECA is immunopathologically linked to vascular injury [2].

Antiendothelial cell antibodies (AECA) are autoantibodies that react with endothelial cells (EC). They show antigen specificity, the ability to attach to surface or intracellular components, and a variety of functional effects. Natural AECA exist, have an anti-inflammatory effects on EC in vitro, and are thought to play a role in the therapeutic efficacy of high-dose intravenous immunoglobulins (IVIG) in the treatment of a range of systemic inflammatory diseases. Many illnesses linked with endothelium disturbance create pathologic AECA in aberrant amounts and composition. For both clinical and experimental research, a variety of tests have been developed to identify and analyze pathologic AECA. Both experimental evidence

and clinical data point to AECA's harmful role: AECA may generate proinflammatory and procoagulant characteristics in EC, as well as produce a variety of disease symptoms in animal models. The frequency and specificity of AECA appear to be illness-specific, and their circulating levels can fluctuate with disease activity. Antigens detected in single illnesses must be identified in order to increase the diagnostic and prognostic value of AECAs detection and to learn more about their mechanisms of action [3].

Antinuclear antibodies (ANAs) are vital laboratory indicators for identifying and diagnosing a wide range of rheumatic disorders (known as ANA-associated rheumatic diseases). The inclusion of ANA positivity as an entry requirement in the 2019 systemic lupus erythematosus classification criteria has increased the importance of ANA testing. Specific ANAs (antibodies to Sm, dsDNA, SSA/Ro60, U1RNP, topoisomerase I, centromere protein B (CENPB), RNA polymerase III, and Jo1) were included in rheumatic disease classification criteria [4].

Antinuclear antibodies (ANAs) in women with pregnancy loss have been confirmed. The presence of mild to high antibody titer of these autoantibodies reveals an autoimmune condition that might threaten the fetus's health in pregnant mothers. ANAs are autoantibodies which have the ability to attach and damage certain components within the nucleus of the cell [5]. Antinuclear antibodies (ANA) are autoantibodies that recognize nuclear and cytoplasmic antigens. Positive ANA is one of the most distinguishing features of autoimmune diseases like systemic lupus erythematosus (SLE), however the correlation between ANA and pregnancy loss is mainly unknown. Positive antinuclear antibodies (ANA) are thought to be a common sign of autoimmunity. Many research attempted to clarify the link between ANA and abortion, however the results were debatable [6]. The pathophysiological mechanism inducing pregnancy loss in women who have had previous miscarriages and have a positive ANA test is yet unclear. Bad oocyte quality, alterations in embryogenesis, and variations in the pattern of uterine blood flow are among the probable causes, according to several research [7]. Considering the probable link between autoantibodies and miscarriage, as well as the high number of couples suffering miscarriage of unknown etiology, it is necessary to establish if ANA may be used as a biomarker for a miscarriage caused by an immunological reaction or immunological origin [8].

2. Materials and Methods

2.1 Subjects

This study's group classification enrolled subjects. Thirty threatened abortion patients, thirty pregnant women, and thirty non-pregnant women visited the Gynecologist at a specialized medical clinic in Babylon province, all of whom were between the ages of 20 and 35.

2.2 Reagents and procedures

Serum autoantibodies was determined by using the commercial ELISA kits for Both AECA (BT LAB, Manufacturer in Shanghai, China) and ANA (SUNLONGBIOTECH, Manufacturer in Hangzhou, China). Principle, procedure, and reagents preparation were prepared according to the manual instructions of manufacturer’s.

2.3 Statistical analysis

Statistical processes and data presentation were performed using SPSS version 24. To examine for differences among study groups, descriptive statistics, Chi-squared test, also Cross tabulation were employed.

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3. Results

This study included 30 participants for each study group, as shown in table (1), the group classification, frequency numbers, and percent of this study enrolled subjects demonstrated table (1).

Table (1): Classification of the study groups.

Study groups	Frequency No.	Percent %
Threatened Abortion	**30**	**33.3**
Pregnant women	**30**	**33.3**
non-pregnant women	**30**	**33.3**
Total	**90**	**100.0**

Table (2) shows the distribution of studied demographical and clinical characteristic variables, patients information such as age, Number of previous abortions and Number of children, were collected for threatened abortion patients, pregnant women as positive control and non-pregnant women as negative control. The majority of participants in the patients group (36.7 %) were all in the age category (24 - 27) years, whereas the majority of participants in the healthy controls group (36.7 % of PC, and 26.7 % of NC) were in the age category (20 - 23) years. However, the difference in age between study groups was statistically non- significant (p-value=0.557). Between the three groups, there was no statistically significant variation in the number of children categories (p-value=0.595). The same table shows that significance differences at p-value (0.015) for number of previous abortions.

Table (2): Distribution of Studied Groups according to Demographical and clinical Characteristics.

		Threatened Abortion patients		Controls				X^2 p-value
				Pregnant women		Non-pregnant women		
		F	%	F	%	F	%	
Age	20 - 23	10	33.3	11	36.7	8	26.7	X^2= 2.077 P= 0.557 N.S
	24 -27	11	36.7	7	23.3	8	26.7	
	28 - 31	5	16.7	6	20.0	6	20.0	
	32 - 35	4	13.3	6	20.0	8	26.7	
	Total	30	100.0	30	100.0	30	100.0	
Number of previous abortions	0	26	86.7	30	100.0	30	100.0	X^2= 8.372 P= 0.015 Sig.
	1	3	10.0	0	0	0	0	
	2	1	3.3	0	0	0	0	
	Total	30	100.0	30	100.0	30	100.0	
Number of children	0	10	33.3	13	43.3	9	30.0	X^2= 4.605 P= 0.595 N.S
	1	3	10.0	9	30.0	2	6.7	
	2	8	26.7	2	6.7	8	26.7	
	3	6	20.0	1	3.3	5	16.7	
	4	2	6.7	2	6.7	4	13.3	
	5	1	3.3	3	10.0	1	3.3	
	6	0	0	0	0	1	3.3	
	Total	30	100.0	30	100.0	30	100.0	

X2:Chi-Square, Sig.: Significance N.S: Non Significance, H.S: highly significance, p-value≤ 0.05

The AECA and ANA autoantibodies were distributed in table (3) according to study groups, AECA positive frequency in TA group was (13.3 %) with a significant difference (p= 0.015), the ANA positive frequency in TA group was (10.0 %) with a significant difference (p= 0.045).

Table (3): Evaluation of ANA and AECA autoantibodies according to study groups.

		threatened abortion patients		Controls				X^2 p-value
				pregnant women (positive control)		Non- pregnant women (negative control)		
		F	%	F	%	F	%	
AECA	Positive	4	13.3	0	0	0	0	X2= 8.372 P = 0.015 Sig
	Negative	26	86.7	30	100	30	100	
	Total	30	100.0	30	100	30	100	
ANA	Positive	3	10.0	0	0	0	0	X2= 6.207 P = 0.045 Sig
	Negative	27	90.0	30	100	30	100	
	Total	30	100.0	30	100	30	100	

X^2:Chi-Square, Sig: significance, p-value≤ 0.05

Furthermore, table (4) expressed the relationship by cross tabulation method between AECA and ANA parameters according to study groups classification to find out how two different variables are related to each other.

Table (4): Relationship between AECA and ANA according to study groups.

Study groups			ANA		Total
			Positive	Negative	
Threatened abortion	AECA	Positive	0	4	4
		Negative	3	23	26
	Total		3	27	30
Pregnant women	AECA	Negative		30	30
	Total			30	30
Non pregnant women	AECA	Negative		30	30
	Total			30	30
Total	AECA	Positive	0	4	4
		Negative	3	83	86
	Total		3	87	90

In Table (5) below Pearson's correlation was applied for the data of this study to detect any association between the studied parameters. It seems to be that age highly significantly linked to number of children of TA patients (corr= 0.616, P= 0.001), while other parameters (Number of previous abortion, Pregnancy period in weeks, AECA and ANA) showed non-significant relationships.

Table (5): Pearson correlation (2-tailed) among Threatened abortion patients studied variables.

Threatened abortion patients		Number of previous abortion	Number of children	AECA	ANA
Age	Pearson C.	0.109	0.616*	-0.058-	0.033
	Sig.	0.566	0.001	0.760	0.863
Number of previous abortions	Pearson C.		0.034	0.144	0.123
	Sig.		0.859	0.447	0.519
Number of children	Pearson C.			0.113	-0.231-

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	Sig.			0.552	0.220
AECA	**Pearson C.**				-0.131-
	Sig.				0.491

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

4. Discussion

Although the pathogenic function of AECA has been hotly contested, there is general agreement that, whatever the mechanism behind inappropriate AECA production, these autoantibodies may cause endothelinal cell activation and/or damage, contributing to vascular pathology in a variety of illnesses. AECA stimulate a dose-dependent proinflammatory and procoagulant phenotype in endothelial cells, upregulating the expression of adhesion molecules (E-selectin, intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1) and tissue factor), stimulating the proinflammatory cytokines (TNFα, interleukin-1 (IL-1), IL-6, and IL-8) and chemokines (monocyte chemotactic protein (MCP)-1), and triggering the cleavage/release of membrane heparin sulfate [3]. In undifferentiated connective tissue disease, autoimmune imbalance and vascular injury might cause complications during pregnancy. Patients with undifferentiated connective tissue disease with a considerably higher concentration of anti-endothelial cell antibody have also been shown to have endothelial cell injury [9].

Autoantibody positivity in RSA patients was reported to be 12 % for ANA and 24 % for AECA, according to a research by [10]. This finding is consistent with our findings to some extent, as ANA 10% and AECA 13.3% were observed to be associated with immunological failure of pregnancy leading to miscarriage when compared with control group; these autoantibodies were found to be correlated with immunological failure of pregnancy leading to abortion, AECA is immunopathologically linked to vascular injury [10].

Antinuclear antibodies are autoantibodies that target nuclear and cytoplasmic antigens and are seen in rheumatic diseases like systemic lupus erythematosus. Positive ANA profiles can appear in otherwise healthy people, indicating an early, undifferentiated stage of certain rheumatic illnesses. These asymptomatic people may remain in this state indefinitely, or they may develop full-fledged illness in months or even years. Despite discrepancies in other investigations, some studies have shown that patients with unexplained recurrent pregnancy loss had increased ANA levels when compared to healthy controls. Recent research has found that repeated pregnancy loss patients had a considerably greater percentage of ANA positive, as well as a link between ANA positivity and an increased risk of recurrent pregnancy loss [11]. Unlike well-known autoantibodies like antiphospholipid antibodies, it's uncertain whether ANAs cause direct harm to embryonic and placental development or serve as a sign of immunological resistance. Despite the discovery of ANAs in follicular fluid and embryos in ANA seropositive women and their link to lower reproductive outcomes by [12], the particular pathways remain unknown. The exact pathophysiology for ANA in early pregnancy loss is not fully explained: Antinuclear antibodies reduce oocyte quality as well as embryogenesis, activate the intraplacental complement cascade, and cause immunecomplex accumulation in placental tissue [13]. Another study indicated that ANAs can affect pregnancy outcomes by deposition an ANA immune complexes at local placental tissue, which activates the complement cascade and causes tissue damage [14].

[15] discovered the frequency of positive ANA tests in healthy controls (0/15) and patients with threatened abortion (3/45), corresponding to our findings of (0/60) for controls and (3/30) for the TA group.

Autoantibodies are formed when the immune system's self-tolerance mechanism fails. The link between autoantibodies and infertility and miscarriage, with or without a systemic disease, is not well established in

the literature. Antithyroid antibodies (anti-thyroglobulin and anti-thyroperoxidase), antiphospholipid antibodies (anti-cardiolipin, anti-2-glycoprotein-I, and lupus anticoagulant), antispermatozoa antibodies, anti-endomysium, anti-DNA, and antinuclear antibodies (ANA) are the most researched autoimmune markers linked to reproductive issues [16].

5. Conclusion

Regarding to these results, we conclude that This immunological tests (AECA as well as ANA) have a significant role in prognosis and/or diagnosis of threatened abortion. Therefore, we can benefit from Examination AECA and ANA to diagnose or predict a threatened miscarriage.

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