Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://doi.org/10.21608/zumj.2024.274116.3223 Volume 31, Issue 1.1, JAN. 2025, Supplement Issue
Manuscript id ZUMJ-2403-3223
Doi 10.21608/zumj.2024.274116.3223

Original Article

The Role of Anti-Nuclear Antibodies in Recurrent Abortion of Immunological Origin

Amira El-Sayed Masoud El-Baz Ahmed[1*], Mohamed Abdel-Moniem Ibrahim[1], Amal Ahmed Ali Zidan[2], Ahlam Ahmed Mohamed Basha[1]

[1]Department of Obstetrics & Gynecology, Faculty of Medicine, Zagazig University, Egypt.
[2]Department of Clinical Pathology, Faculty of Medicine, Zagazig University, Egypt.

***Corresponding author:**
Amira El-Sayed Masoud El-Baz Ahmed

E-mail:
dramiraelsayed2019@gmail.com

Submit date 08-03-2024
Revise Date 28-03-2024
Accept date 23-04-2024

ABSTRACT

Background: Recurrent pregnancy loss (RPL) is the happening of two or more miscarriage events before the 20th week of pregnancy. This research aimed to assess the role of ANA in recurrent pregnancy loss of immunologic origin in Egyptian pregnant females.

methods: This was case-control research performed on 70 women in their first trimester of pregnancy at Zagazig University Hospitals. Patients were subdivided into 2 groups.

Results: There was a significant association between RPL and both positivity of ANA (OR=4.337, p=.076) and parity (OR= 1.62, p=.054) at significance level of 0.1 which is acceptable as non-standard p-value, that means a female with positive ANA test has 4.3 times chances to have RPL compared to a female with negative ANA test, also females with each one increase in number of previous successful given births (parity) has a correspondent 1.621 times increase of probability to have recurrent pregnancy loss

Conclusions: (ANA) has an important role in prognosis recurrent pregnancy loss. Therefore, we can benefit from Examination ANA to diagnose or predict a threatened miscarriage.

Key words: Anti-Nuclear Antibodies; Recurrent Miscarriage

INTRODUCTION

Recurrent pregnancy loss (RPL) defined by the American Society of Reproductive Medicine (ASRM) is the happening of two or more miscarriage events pre the twentieth week gestation. It occurs due to a wide variety of causes (translocation, endocrine disorders, immunological disorders, and uterine disorders known to be responsible for 50% of cases of (RPL) [1].

It has long been known that immunology has a negative effect on reproductive outcomes, and immunologic pathways have been shown to play a role in RPL. Growing interest has been shown in the roles that autoantibodies play in RPL in last year [2].

A class of autoantibodies known as antinuclear antibodies (ANA) target antigens found in the nucleus and cytoplasm. Although the correlation between ANA and RPL remains largely unknown, the presence of antibodies against ANA is one of the most distinguishing features of autoimmune diseases like systemic lupus erythematosus (SLE). The relationship between ANA and RPL has been the subject of numerous studies for the past thirty years; however, the findings remain extremely contentious [2].

An elevated ANA titer (greater than 1:160) was associated with RPL. Comparing to low ANA concentrations, high ANA concentrations might cause higher maternal immune response and result in decreased favorable pregnancy outcomes [3].

The objective of this research was to assess the role of ANA in recurrent pregnancy loss of immunologic origin in Egyptian pregnant females.

METHODS

Seventy women in the initial stage of conception were the subjects of this case-control research in Zagazig University

Hospitals. Patients were divided into two groups; RPL group: recurrent pregnancy loss group included 45 women and Control group: women with no recurrent pregnancy loss & included 25 women.

Sample size justification

Assuming that the proportion of ANA+ cases in control group is 0.16 and proportion of ANA+ cases in recurrent pregnancy loss group is 0.50 at eight percent power and ninety- nine CI The approximate sample size will be 66 subjects, 22 in control group and 44 in the recurrent pregnancy loss group by using the G- Power program.

Inclusion Criteria for study groups: Between the ages of twenty and forty-five, the gestational age ranges between six and twenty weeks (as determined by an ultrasound examination and a reliable last menstrual period during the first trimester), Singleton pregnancy viability, Remaining membranes, Maternal BMI between (18-30kgandm2) and Previous 2 or more miscarriages pre the 20th week of pregnancy.

Exclusion Criteria for study groups: Multiple pregnancy, cases with ROM and presence of any well-known anatomical, genetic, or endocrinal factors that can lead to miscarriage.

Patients were subjected to

Complete history taking: History including demographic data like Age, Name address, &material state consanguinity, number of Parity, Detailed history of previous pregnancy losses. Presence of HTN, DM and any other chronic or immunological diseases and medication administration. **Examination:** General examination, Abdominal and local clinical examination: Abdominal inspection: for any abnormality like swelling, scares, organomegaly.

Investigations: Abdominal US to assure viability of the baby, general investigations to assure the general wellness of the pregnant participant and endocrinal investigations to assure absence of any endocrinal risk factors of RPL, like hypothyroidism.

Anti-nuclear antibodies (ANAs)

ANA Assay (FANA) test: A patient's blood sample obtained from the study participants and sent for ANA level analysis in Zagazig university hospital labs, A FANA test report includes a negative or positive interpretation of presence, ANA titre 1:160 is minimum titer ratio and patterns seen in antibodies during the test. Homogenous, Speckled, Centromere or Nucleolar [4].

Follow up: 70 participants of this study were followed up to determine the outcome of pregnancy either by successful delivery or by miscarriage.

Ethical Consideration

The study protocol received approval from Zagazig University's Institutional Review Board. Written informed give his approval was obtained from every participant who agreed to participate in the research. At each stage of the research, confidentiality and individual privacy were confirmed. (IRB: 9171/11-4-2022)

STATISTICAL Analysis

Using Microsoft Excel, data obtained over time, including basics clinical examinations, laboratory analyses, and outcome measures, were coded, provided, and analyzed. SPS version 20.0 (Statistical Package for the Social Sciences) was subsequently utilized to import the data. Software for analysis (Statistical Package for the Social Sciences). According to the type of data qualitative represented as number and percentage, quantitative continues group represent by mean ± SD, the following tests were used to test differences for significance؛ logistic regression analysis and Odds Ratio calculation The p-value thresholds for significant and highly significant results were set at 0.05 and 0.001, respectively. The collated data was subsequently subjected to statistical analysis. The statistical tests and parameters utilized were as follows: Standard deviation (SD) and mean

RESULTS

A lack of statistically significant variation (p=0.896) was observed in the age categories of the two groups (Table 1).

Those in the recurrent pregnancy loss group had considerably greater miscarriage rate compared to control group (p<0.001) (Table 2).

Recurrent pregnancy loss group had non-significantly slightly higher prevalence of positive ANA test for all its levels compared to control group at significance level of .05.

However, since the actual p value here is .061 this can be considered significant if we are liberal to set the p value to 0.1 (Table 3).

No substantial distinction was observed in terms of outcome between the two groups. (p=0.090) (Table 4).

There was a significant association between RPL and both positivity of ANA (OR=4.337, p=.076) and parity (OR= 1.62, p=.054) at significance level of 0.1 which is acceptable as non-standard p-value, that means a female with positive ANA test has 4.3 times chances to have RPL compared to a female with negative ANA test, also females with each one increase in number of previous successful given births (parity) has a correspondent 1.621 times increase of probability to have recurrent pregnancy loss (Table 5).

Table (1): Comparison between the two groups regarding age.

		RPL group (No. = 45)		Control group (No. = 25)		Test value	P-value
		No.	%	No.	%		
Age groups	**20- <30 years**	22	48.9%	13	52.0%	X^2= 0.219	0.896
	30- <40 years	15	33.3%	7	28.0%		
	≥40 years	8	17.8%	5	20.0%		
Age (years)	**Mean± SD**	30.76± 7.43		31.04± 8.13		Z_{MWU}= 0.080	0.926
	Median	31.0		29.0			
	Range	20.0 – 44.0		20.0 – 45.0			

p≤0.05 is considered statistically significant, p≤0.01 is considered high statistically significant, SD= standard deviation, *Mann-Whitney test and Chi-Square Test

Table (2): Comparison between the two groups regarding miscarriage.

		RPL group (No. = 45)		Control group (No. = 25)		Test value	P-value
		No.	%	No.	%		
Miscarriage	**1**	0	0.0%	25	100.0%	X^2= 70.0	**<0.001**
	2	34	75.6%	0	0.0%		
	3	7	15.6%	0	0.0%		
	4	4	8.9%	0	0.0%		
	Mean± SD	2.33± .64		1.00± 0.0		Z_{MWU}= 7.528	**<0.001**
	Median	2.0		1.0			
	Range	2.0 – 4.0		1.0 – 1.0			

p≤0.05 is considered statistically significant, p≤0.01 is considered high statistically significant, SD= standard deviation, *Mann-Whitney test and Chi-Square Test

Table (3): Comparison between the two groups regarding anti-nuclear antibodies.

		RPL group (No. = 45)		Control group (No. = 25)		Test value	P-value
		N.	%	N.	%		
ANA	**Negative**	33	73.3%	23	92.0%	X^2= 3.500	.061
	Positive	12	26.7%	2	8.0%		

p≤0.05 is considered statistically significant, p≤0.01 is considered high statistically significant

Table (4): Comparison between the two groups regarding outcome.

		RPL group (No. = 45)		Control group (No. = 25)		Test value	P-value
		No.	%	No.	%		
Outcome	Miscarriage	11	24.4%	2	8.0%	X^2= 2.874	0.090
	Delivery	34	75.6%	23	92.0%		

p≤0.05 is considered statistically significant, p≤0.01 is considered high statistically significant, SD= standard deviation, * Chi-Square Test

Table (5): Multivariate logistic regression analysis for factors associated with RPL.

Parameters	B	S.E.	Wald	P-value	Odds ratio (OR)	95%CI	
						Lower limit	Upper limit
Age	-.018	.017	1.125	.289	.982	.950	1.015
ANA	1.467	.826	3.158	.076	4.337	.860	21.873
Parity	.483	.251	3.711	.054	1.621	.992	2.650

DISCUSSION

Our finding showed that recurrent pregnancy loss group had significantly higher miscarriage rate contrast to control group (p<0.001).

That supported by Merdas et al. [5] who reported there was significance increase of number of previous miscarriages in threatened miscarriage group compared to healthy pregnancy group and normal un pregnant women group, p-value (0.015).

We showed that 12 women (26.7%) in recurrent pregnancy loss group and 2 women (8.0%) in control group were ANA positive. This difference is significant at the 0.1 significance level, in this situation increasing the number of cases will be beneficial as it will reveal the hidden significance difference that may be masked by low number of participants.

Merdas et al. [5] also, reported a significant difference in ANA positive frequency in women with threatened miscarriage (TA) compared to those with no recurrent pregnancy loss. However, a multivariate logistic regression analysis showed a significant relationship between recurrent pregnancy loss (RPL) and ANA positivity, with increasing number of successful given births having a significant association with RPL.

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 Ying et al. [6].

Abuzeid et al. [7] who reported that there was significance between age and RPL.

The serum of patients who have experienced both unexplained and explained pregnancy losses has been found to contain a significant amount of low-titer ANA, according to several studies. Nevertheless, the meanings of the results remain unreliable. An increased prevalence of antinuclear antibodies was identified in patients with autoimmune disease by E. M. Tan and others. Nevertheless, no evidence suggested that they were more prevalent among patients who had experienced recurrent pregnancy loss or infertile [8].

Variations in ANA titers have little clinical significance in the context of autoimmune disease, despite their importance in test interpretation. In one study of 125 patients with a positive ANA but no other indications of connective tissue disease, titers exceeding 1.40 were detected in 32% of cases, exceeding 1.80 were detected in 13%, and exceeding 1.320 were observed in only three percent of patients. [9].

Molazadeh et al. [10] A total of 74 of them out of 560 patients (13.21%) who had recurrent miscarriage were found to have ANAs, compared to only 5 out of 560 controls (0.9%) ($p<0.001$). An overall analysis showed that thirty-eight percent of positive cases contained low-positive results (1.40-1.80), while forty-six percent and sixteen percent of cases contained moderate titres (1.160-1.320) and high titres (>1.640), respectively. In the end, from microscopic examination of ANA patterns, it was found that approximately 50% of positive cases exhibited antibodies targeting the DNA-histone complex, which is linked to systemic lupus erythematosus disease. Abuzeid et al. [7] who reported that ANA is associated with miscarriage as 27.0% of recurrent pregnancy loss had ANA+ while none of the control group had ANA+.

Also, our results were supported by Hamadi & Lafta [11] in which 29% of the patients were positive for antinuclear antibodies (ANA), while the control subjects had negative results for these autoantibodies.

Molazadeh et al. [10] who antinuclear antibodies are frequently detected in women with no explain recurrent miscarriages without explanation, demonstrating that an autoimmune disorder may play a role in miscarriage, at least in a subset of patients.

ANA mechanism in fetal rejection has not yet been adequately identified. The rate of ANA is prevalent in women with recurrent miscarriage with uncertain causes [12].

Regarding the fact that the cause of recurrent spontaneous miscarriage is unknown in about 50% of cases, immunologic factors have been implicated in these cases and several findings have been obtained about the role of immunological factors. Also, immunological therapies for recurrent spontaneous miscarriage are increasing. Accordingly, the identification and investigation of the role of immunological factors in recurrent spontaneous miscarriage is of great importance [13].

Ticconi et al. [14] who reported that Pregnancy was associated with forty-five ANA-positive women and 41 ANA-negative women. The outcome of the next pregnancy did not exhibit any correlation with the pre-pregnancy ANA status. While miscarriages happened in seven out of twenty-two ANA+ women (31.8percent) during the seventh week of pregnancy monitoring for ANA. None of the twenty-three ANA+ women who transitioned to ANA− experienced miscarriages during their pregnancies beyond the twentieth week.

Antinuclear antibodies (ANAs) are a distinct category of autoantibodies that are capable of destruction specific molecules located within the cellular nucleus. The precise mechanism by which antinuclear antibodies (ANAs) induce miscarriage remains poorly understood; however, a hypothesis proposes that these antibodies induce inflammation of the uterus, rendering it unsuitable for implantation of embryos [15].

CONCLUSIONS

(ANA) has a significant role in prognosis and/or diagnosis of recurrent pregnancy loss. Therefore, we can benefit from Examination ANA to diagnose or predict a threatened miscarriage.

POINTS OF STRENGTH

Inclusion of age and parity as confounder was a strength point as they eliminated any confusion that confounders served as co-predictors, if weren't used. Following up of cases till delivery was another strength point as it showed the real effect of ANA beyond week 20.

LIMITATIONS

The only limitation to this study was the low number of participants was a limitation as it hidden the two groups' significant variance as indicated by the p value was 0.067 and increasing the number of cases affects it.

Conflict of interest statement: The authors declared that there were NO conflicts of Interest.

Disclosure: The authors have no financial interest to declare in relation to the content of this article.

REFERENCES

1. **KHALIFE, Dalia; GHAZEERI, Ghina.** Review of current guidelines for recurrent pregnancy loss: new strategies for optimal evaluation of women

who may be superfertile. In: Seminars in perinatology. WB Saunders, 2019. P. 105-115.
2. **PILLARISETTY, Leela Sharath; MAHDY.** Recurrent pregnancy loss. 2020.1-35.
3. **ANUPRIYA, A.,** Antinuclear antibodies in patients with unexplained recurrent miscarriages. Asian J Pharm Clin Res, 2017, 10.8: 256-259.
4. **KUMAR, Rajeev; WYMAN, Charles E.** Does change in accessibility with conversion depend on both the substrate and pretreatment technology? Bioresource Technology, 2009, 100.18: 4193-4202.
5. **MERDAS, Ahmed H.; IQBAL.** AECA and ANA in threatened miscarriage women in Babylon province. AECA, 2022, 140.01.
6. **YING, YingA** further exploration of the impact of antinuclear antibodies on in vitro fertilization–embryo transfer outcome. American Journal of Reproductive Immunology, 2013, 70.3: 221-229.
7. **ABUZEID, Nadir.** Assessment of selective auto antibodies in pregnant women with spontaneous recurrent miscarriage and non-miscarriage in al-gezira state-sudan. Sudan Medical Laboratory Journal, 2022, 10.1: 23-32.
8. **TAN, E. M.** Range of antinuclear antibodies in "healthy" individuals. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology, 1997, 40.9: 1601-1611.
9. **SLATER, Cindi A.; DAVIS, Roger B.** Antinuclear antibody testing: a study of clinical utility. Archives of Internal Medicine, 1996, 156.13: 1421-1425.
10. **MOLAZADEH, Morteza; KARIMZADEH.** Prevalence and clinical significance of antinuclear antibodies in Iranian women with unexplained recurrent miscarriage. Iranian Journal of Reproductive Medicine, 2014, 12.3: 221.
11. **HAMADI, Ghaneemah Malik; LAFTA.** Immunological parameters of recurrent miscarriages among women in Thi-Qar province. Journal of Medicine and Life, 2022, 15.5: 635.
12. **SHOENFELD, Yehuda.** Autoantibodies and prediction of reproductive failure. American Journal of Reproductive Immunology, 2006, 56.5-6: 337-344.
13. **ROUMANDEH, Narges; ZARE.** Immunology of Recurrent Spontaneous Miscarriage. Sarem Journal of Medical research, 2018, 3.2: 121-126.
14. **TICCONI, Carlo.** Antinuclear autoantibodies in women with recurrent pregnancy loss. American Journal of Reproductive Immunology, 2010, 64.6: 384-392.
15. **WALIA, Gagandeep Kaur.** Immuno-molecular etiology of recurrent pregnancy loss and the anthropological perspective. International Journal of Human Genetics, 2008, 8.1-2: 227-235.

Citation

El-Baz Ahmed, A., Ibrahim, M., Ali Zidan, A., Mohamed Basha, A. The Role of Anti-Nuclear Antibodies in Recurrent Abortion of Immunological Origin. *Zagazig University Medical Journal*, 2025; (333-338): -. doi: 10.21608/zumj.2024.274116.3223