

Is there a role of antisperm antibodies in women with unexplained infertility - a Turkish pilot cross sectional case control study

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Abstract

Aim: Although some studies indicate that the presence of antisperm antibody (ASA) is important in the etiology of female infertility, this remains a controversial issue. The aim of this study was to determine whether the presence of ASA was important or not in primary and secondary unexplained infertility patients.

Materials and Methods: Female patients (n = 90) were divided into three groups as follows: the primary infertility group (n = 30), the secondary infertility group (n = 28) and a control group (n = 32). In addition to laparoscopic evaluations, the presence of ASA in the study groups was also examined in serum and peritoneal washing liquid. The ASA - immunoglobulin G was measured using the Enzyme-Linked ImmunoSorbent Assay (ELISA) method with a qualitative measurement kit.

Results: When the demographic data were analyzed, it was observed that the study groups were homogeneous in terms of age and period of infertility. The results of the study demonstrated that, when compared to the secondary infertility and control groups, the ASA positivity in the serum in the primary infertility group was significant (p < 0.05). However, when the groups were compared, there was no difference in terms of ASA positivity in peritoneal washing fluid (p > 0.05).

Conclusion: This study showed that the presence of ASA in serum may be an impact on the etiology of unexplained primary infertility.

Keywords: Antisperm antibody; immunoglobulin G; primary infertility; secondary infertility; unexplained infertility

INTRODUCTION

According to the latest international glossary from the World Health Organization, unexplained infertility (UI) is defined as the absence of conception after 1 year of unprotected intercourse, and which is not explained by anovulation, tubal pathology, bad semen quality or any other known cause of infertility. There are two types of infertility. Primary infertility refers to couples who have not become pregnant after at least one year coitus without using contraceptive methods while secondary infertility refers to couples who have been able to get pregnant at least once in the past, but now are unable to conceive again (1,2).

UI represents about 25% to 40% of all infertility. Scientists have done a great deal of research on the unexplained causes of infertility. Now, we know that autoimmunity and alloimmunity have a very important role in UI (for example antisperm antibodies (ASAs), antiovarian antibodies, anti-zona pellucida antibodies and antiphospholipid antibodies) (3). ASAs are one of the most important molecules studied in this situation (4). ASAs form because of the

autoantigenic and isoantigenic potential of spermatozoa (5). ASAs can be isolated in several tissues, for example, in semen fluid and on the spermatozoa surface, in the blood sera of men and women, in the fallopian tube fluid, the cervical mucus, and in the follicular and peritoneal fluid of women (6).

According to published literature, immune reactions caused by ASAs are an important cause of unexplained infertility in men and women (7,8). But some studies have shown that this remains a controversial topic in the field of female infertility (9).

There is a debate about the role of ASAs in pregnancy formation. The main purpose of this study was to investigate the presence and importance of ASAs in unexplained primary and secondary infertility.

MATERIALS and METHODS

Patients

This study performed in the obstetrics and gynecology clinic between January 2017 and December 2019. Written informed consent was obtained from each participant

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involved in the study. The recommendations of the 'Declaration of Helsinki' were taken into consideration. The study was approved by the ethics committee (Ethics Committee Number: 33216249-903.99-05/15).

This study was performed at a tertiary health center and involved 90 patients in total. Thirty of them suffered primary infertility (PIG), 28 of them suffered secondary infertility (SIG) and 32 of them were control group (CG) patients. The control group consisted of patients who had no infertility problems and preferred laparoscopic tubal ligation for contraception. The other groups included in the study consisted of patients presenting to our outpatient clinic with primary and secondary infertility.

Initially, a detailed anamnesis was obtained from couples who presented with infertility. Then, a physical examination and some routine tests were performed to investigate the patient etiology: thyroid-stimulating hormone (TSH), free thyroxine (fT4), prolactin (PRL), follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2) levels of women at the second or third day of menstruation, pelvic ultrasonography, hysterosalpingography and a spermiogram. Diagnostic laparoscopy was recommended for patients whose routine tests were normal to clarify the etiology. We eliminated patients who had endometriosis or peritoneal adhesions after diagnostic laparoscopy, and who had abnormal routine test results. Inclusion and exclusion criterias for study groups are summarized in Figure 1.

On the day of surgery, we collected blood samples from the brachial vein and peritoneal washing fluid from the pouch of Douglas. Tissue samples were maintained under appropriate laboratory conditions and the presence of ASA was investigated in both samples using the Enzyme-Linked Immuno Sorbent Assay (ELISA) method.

Examination of Laboratory Tests

Blood samples were taken from the patients after 8 hours of fasting, at the early follicular phase (between the third and the fifth days of menstruation). Venous blood was taken from the brachial vein in the early morning (between 08:00 to 10:00 hours), and centrifuged immediately. The sera were kept at -80°C until testing.

FSH, LH, E₂, TSH, fT4 and PRL levels were determined using a chemiluminescence immunoassay method (Centaur XP, Siemens Healthcare Germany).

The ASA - immunoglobulin G (ASA - IgG) was measured using the ELISA method with a SunredBio kit (Catalogue No:201-12-1858; Shanghai Sunred Biological Technology Co., Ltd., China). In this study, a qualitative measurement kit was used. Accordingly, optical density values less than 300 nanometers were evaluated to be a negative result and values higher than 300 nanometers were evaluated to be a positive result.

Statistical Analysis

SPSS 22.0 software was employed for the statistical analysis (SPSS Inc., Chicago, IL). Mean and standard deviation (mean \pm SD) descriptive statistical methods were used. The results of homogeneity (Levene's test) and normality (Shapiro-Wilk test) were used to decide the statistical methods for comparing the study groups. Among normally distributed groups with homogeneous variances, dependent groups were compared using the Student's t-test. According to the test results, parametric test assumptions were not available for some variables; therefore, the independent groups were compared using the Mann Whitney-U test. Categorical data were analyzed using Fischer's exact test and the chi-square test. A $p < 0.05$ level was considered to be statistically significant.

Primary Infertility Group (n = 30)	Secondary Infertility Group (n = 28)	Control Group (n = 32)
inclusion criteria:	inclusion criteria:	inclusion criteria:
- matching the definition of primary infertility	- matching the definition of secondary infertility	- had no infertility problems
- had normal physical and ultrasonographic examination	- had normal physical and ultrasonographic examination	- preferred laparoscopic tubal ligation for contraception
- had normal 3 rd day hormone profile	- had normal 3 rd day hormone profile	
- had normal spermiogram	- had normal spermiogram	
- had normal hysterosalpingography	- had normal hysterosalpingography	
exclusion criteria:	exclusion criteria:	
- had endometriosis	- had endometriosis	
- had peritoneal adhesions	- had peritoneal adhesions	
- had abnormal hormone or spermiogram or hysterosalpingography results	- had abnormal hormone or spermiogram or hysterosalpingography results	

Figure 1. Flowchart of inclusion and exclusion criterias to the study groups

RESULTS

Table 1 summarizes the demographic characteristics of the study population. The mean age ($p = 0.061$) was similar in the SIG and PIG. Gravida was higher in the CG than SIG (3.2 ± 0.8 vs 1.1 ± 0.3 at $p = 0.001$, respectively). Abortus ($p = 0.867$) was similar in the SIG and CG. Infertility time ($p = 0.519$) was similar in the PIG and SIG. In addition, there was no significant difference between the 3 groups for alcohol use, smoking and body mass index (BMI).

Table 2 summarizes the ASA results of the study groups. There was no ASA positivity in the SIG. Only 4 patients

had ASA positivity in peritoneal washing liquid from the CG. In the PIG, 6 patients had ASA positivity in serum and 4 patients had ASA positivity in peritoneal washing liquid. When the SIG and CG were compared, the ASA positivity in serum in the PIG was significant ($p < 0.05$). But, when compared to the SIG and CG, the ASA positivity in peritoneal washing liquid in the PIG was not significant ($p > 0.05$).

Table 3 reflects the hormone results of the patient groups included in the study. When the groups were compared, there was no statistical difference between the hormone results.

Table 1. Demographic data of the patients participating in the study

	Primary infertile (n:30)	Secondary infertile (n:28)	Control (n:32)	p1	p2	p3
Age (mean±SD)	29.1±4.7	31.8±4.7	34.0±4.0	0.061	0.001	0.163
Gravida	0	1.1±0.3	3.2±0.8	0.001	0.001	0.001
Parity	0	0.7±0.4	2.8±0.9	0.001	0.001	0.001
Abortus	0	0.4±0.5	0.4±0.5	0.001	0.001	0.867
Infertility time (year)	5.3±3.1	6.0±2.6	0	0.519	0.001	0.001
BMI (kg/m ²)	23.57 ± 2.83	24.04 ± 1.96	25.06 ± 1.98	0.124	0.140	0.366
Smoking	13.5 ± 1.9	12.6 ± 1.7	13.6 ± 1.5	0.953	0.999	0.952
Alcohol use	3.2 ± 1.1	2.2 ± 0.9	3.3 ± 0.9	0.964	0.999	0.971

SD: Standard Deviation; BMI: Body Mass Index; PIG: Primary Infertility Group; SIG: Secondary Infertility Group; CG: Control Group
p1: Comparison of PIG and SIG groups; p2: Comparison of PIG and CG groups; p3: Comparison of SIG and CG

Table 2. The ASA positivity of serum and peritoneal washing liquid for 3 groups

	Primary infertile (n:30)	Secondary infertile (n:28)	Control (n:32)	p1	p2	p3
Serum	6	0	0	0.005*	0.003*	0.065*
Peritoneal washing liquid	4	0	4	0.299*	0.448*	0.299*

ASA: Antisperm Antibody, PIG: Primary Infertility Group; SIG: Secondary Infertility Group; CG: Control Group; *Chi-square test results
p1: Comparison of PIG and SIG groups; p2: Comparison of PIG and CG groups; p3: Comparison of SIG and CG

Table 3. Hormone results of the patients participating in the study

	Primary infertile (n:30)	Secondary infertile (n:28)	Control (n:32)	p1	p2	p3
FSH (μU/mL)	6.71 ± 0.72	7.12 ± 0.41	6.92 ± 0.52	0.322	0.215	0.292
LH (μU/mL)	4.52 ± 0.55	4.62 ± 0.62	4.66 ± 0.31	0.411	0.358	0.365
E2 (μU/mL)	65.16 ± 1.25	67.31 ± 0.55	66.24 ± 0.92	0.252	0.311	0.341
PRL (μU/mL)	11.44 ± 0.62	10.63 ± 0.54	11.37 ± 0.66	0.962	0.952	0.957
TSH (μU/mL)	2.69 ± 0.32	2.52 ± 0.51	2.72 ± 0.44	0.371	0.414	0.369
ft4 (μU/mL)	0.91 ± 0.12	0.96 ± 0.14	0.90 ± 0.34	0.524	0.531	0.574

PIG: Primary Infertility Group; SIG: Secondary Infertility Group; CG: Control Group; FSH: Follicle-Stimulating Hormone; LH: Luteinizing Hormone; E2: Estradiol, PRL: Prolactin; TSH: Thyroid-Stimulating Hormone; ft4: Free Thyroxine; μU/mL: micro - Unit / milliliter
p1: Comparison of PIG and SIG groups; p2: Comparison of PIG and CG groups; p3: Comparison of SIG and CG

DISCUSSION

When the results of our study were examined, we saw that ASA positivity in peritoneal washing fluid did not have any significance. But ASA positivity in the serum of primary infertile patients was significant when compared with the other two groups.

The prevalence of ASAs in both men and women was less than 2%. In infertile couples, ASAs were present in 5 to 25% of individuals. When the published literature is examined, there is sufficient evidence that ASAs impair fertility in couples with UI. It was demonstrated that ASAs reduce the fertile capacity through some different mechanisms. One of these ASA mechanisms is the secretion of histamine in uterine tissue which causes the expulsion of the implanted embryo (9).

Chang et al. showed that the ASAs on spermatozoa caused lower fertilization rates, and IgG was the major immunoglobulin involved in this study (10). Because of this information we used ASA-IgG in our study.

Somigliana et al. declared that fecundity declines with age, so discriminating between unexplained infertility and age-related infertility becomes more difficult as the woman's age increases. In our study, the mean age of patients in the PIG and SIG were similar, and this situation eliminated age-related infertility in our study (11). Also the infertility period for our patients ($p = 0.519$) was similar in both SIG and PIG. This information shows the homogeneous nature of our study population.

Kamieniczna et al. declared that 4.1% of the serum samples from infertile women were positive for ASA, but they did not classify the study group in terms of primary or secondary infertility (12). In our study, the positivity rate of ASAs in serum was 20% in PIG, and 0% in SIG. The mean ASA positivity rate in our infertility group was 10.3%. Sperm exposure during menses and being a sex worker are risk factors for developing ASA in women (13,14); however, there was no such risk factor in our study population.

Stern et al. compared the presence of ASA in serum and peritoneal washing fluid collected laparoscopically in their studies. They declared that ASA in serum was not correlated in all cases with ASA in peritoneal washing fluid (6). As seen in Table 2, the presence of ASA in the peritoneal washing fluid and serum was not correlated. This result supports the literature.

When the 3 groups in our study were compared, there was no statistically significant difference in ASA positivity in peritoneal washing fluid. However, when the groups were examined in terms of serum ASA positivity, there was a statistically significant difference between the primary infertility group and the other two groups (Table 2; $p_1 = 0.005$ and $p = 0.003$). This result suggests that the presence of ASA in serum has an impact on the etiology of unexplained primary infertility.

In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are the most commonly used treatment methods for infertility problems. Vujisic et al. declared

that the presence of ASAs in men or women was not associated with poorer IVF outcomes (15). Esteves et al. reported that ICSI success is not dependent on the presence of ASA (16). Chang et al. also reported that the ASAs on spermatozoa (IgG) and in female serum (IgM) causes lower numbers of transferred embryos (10). According to the published literature, ASAs can affect IVF success and this is dependent on the subtypes of ASAs.

LIMITATIONS

This study features some limitations. Firstly, there is controversy about the role of ASAs in unexplained infertility in published literature. Secondly, our study population was limited. Future research should be organized with many more patients. Thirdly, we used a qualitative measurement kit and we studied only IgG. Future research should be organized with a quantitative measurement kit and should study IgG, IgM and IgA. Fourthly, in order to fully understand the role of ASAs on unexplained infertility, future research should also examine the other immunological factors (for example, antithyroid antibodies, antiovarian antibodies, anti-zona pellucida antibodies and antiphospholipid antibodies).

CONCLUSION

The fact that ASA - Ig G was found to be significantly positive in the serum of primary infertile patients in our study showed that the presence of ASA should still be considered as an etiological factor. However, there is no clear data about the etiological significance of other immunoglobulins such as Ig M and Ig A. The mechanism by which the presence of ASAs leads to unexplained infertility is still not fully understood. To solve this problem, researchers use assisted reproductive techniques such as IVF and ICSI. If the mechanism by which the presence of antisperm antibodies cause infertility can be solved at a molecular level in the future, such expensive treatments like ICSI and IVF may be replaced by cheaper immunosuppressive treatments.

Competing Interests: The authors declare that they have no competing interest.

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