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Original Article

Analysis of the serum reproductive system related autoantibodies of infertility patients in Tianjin region of China

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Abstract: Object: Reproductive system related autoantibodies have been proposed to be associated with natural infertility. However, large scale systematic analysis of these of antibodies has not been conducted. The aim of this study is to analyze the positive rate of antisperm antibody (ASAb), anti-endometrium antibody (EMAb), anti-ovary antibody (AOAb), anti-zona pellucida antibody (AZP) and anticardiolipin antibody (ACA) in infertility patients in Tianjin region of China. Methods: 1305 male and 1711 female primary infertility patients and 1100 female secondary infertility patients were included in this study, as well as 627 healthy female controls. The above autoantibodies were tested and the positive rates in each group were calculated. Results: the positive rate of ASAb were significantly higher in primary infertility female than that in male, further analysis revealed that primary infertility population all exhibit significant higher positive rate of EMAb, AOAb, AZP and ACA compared with control group. Furthermore, the positive rates of all the antibodies in primary infertility female were significantly higher than those in secondary infertility female. Conclusions: Our study thus indicates that these autoantibodies might be associated with immunological related primary infertility and may have clinical significance in its diagnosis and treatment.

Keywords: Immune, autoantibody, infertility, EMAb, AOAb, AZP, ACA

Introduction

Infertility is a disease in reproductive system characterized by inability to conceive or to produce normal sperm in women or men, respectively. It is a worldwide problem with a 4% increase of incidence rate since the 1980s [1]. Particularly in China, it has been estimated that 10%-15% of the married couples are suffered from infertility. It not only has serious adverse impact on patients' life qualities but also brings about huge social-economic burdens. Thus, exploring the effective ways to diagnose and treat infertility is of vital importance.

Despite that DNA damage, endocrine disorders, genetic factors and environmental factors are associated with its etiology [2-5]; the specific cause of infertility is still obscure and to be determined. Recent studies addressed the great influence of the immune system on women infertility [6-8], which highlighted the urgency of the exploration of reproductive immunology. Yet it has long been clarified that a series of anti-reproductive antibodies exist in the serum of both male and female, which may potentially contribute to both the male and female infertility factors. Among these autoantibodies, antisperm antibody (ASAb) [9, 10], anti-endometrium antibody (EMAb), anti-ovary antibody (AOAb) [11], anti-zona pellucida antibody (AZP) [12] and anticardiolipin antibody (ACA) [13] are believed to potentially interfere with the process of fertilization and implantation, eventually results in implantation failure and abortion. Typically, serious studies have analyzed the possible role of some these autoantibodies in *in vitro* fertilization (IVF) failure; for example, serum EMAb, ACA, AZP and AOAb are often seen in unsuccessful IVF patients and exerts detrimental effect [14-20]. Although these studies sporadically identified their role

Table 1. The positive rate of ASAb in infertile male and female

Sex	Total case (N)	ASAb positive case (N)	Positive rate (%)
Male (primary infertility)	1305	378	28.97[a]
Male healthy control	634	17	2.68%
Female (primary infertility)	1711	595	34.77[b]
Female (secondary infertility	1100	445	40.45[b]
Female healthy control	627	15	2.39

[a]P<0.01, compared with healthy male group; [b]P<0.01, compared with female healthy control.

in the immune infertility, a systematic analysis of the related autoantibodies in large scale clinical samples is needed.

As such, we compared the serum autoantibodies including ASAb, EMAb, AOAb, AZP and ACA of infertility patients with those in normal people in a Chinese population of Tianjin region. Our analysis may provide some supportive information's for the diagnosis of immune infertility.

Patients and methods

Study subjects

We included 3016 patients who suffered from primary infertility and were admitted in the infertility outpatient, department of gynaecology and obstetrics and department of andrology in The Second Hospital of Tianjin Medical University from January 2007 to December 2014. Among these patients, 1305 were male and 1711 were female. Urological and gynecologic examinations were performed to rule out the malformations and other possible diseases in reproductive system. For females, the womb fallopian tube iodine oil radiography and tubal patent test by liquid instillation was carried out to ensure every subject have patency in their oviducts; we also used B-mode ultrasonography to confirm the existence of ovulatory cycle every female subject. For males, individuals with general normal values of the sperm count, sperm motility and viability were qualified. We also included 1100 female who suffered from secondary infertility in this study. All the study subjects were free from systemic lupus erythematosus (SLE) and other rheumatic diseases; none of the subjects was lupus anticoagulant (LA) positive. The blood samples of each group were collected; blood samples of 627 normal females and 634 normal males from Tianjin blood center were used as control. The informed consents were obtained, and whole study was approved by the medical ethical committee of The Second Hospital of Tianjin Medical University with approved number of T2-2006-13.

Detection of autoantibodies

The serum of each blood sample was diluted at 1:100 before the detection. The AOAb, AZP, ACA and EMAb were detected dot immuno-gold filtration assay (DIGFA). The DIGFA kit for EMAb and ASAb was purchased from Kangrun biotechnology (Guangzhou, China), and the DIGFA kits for AOAb, AZP and ACA were purchased from Anqun biotechnology (Shenzhen, China). The DIGFA by using monoclonal antibody for each antigen and colloidal gold labeled with affinity purified goat anti-mouse IgG was developed for the rapid detection of antigens mentioned above. All the experimental procedures were conducted according to the instructions provided by the manufacturer. The positive result showed red colored dots, while no color was observed as the negative result.

Statistical analysis

The data were analyzed by SPSS 19.0 software (IBM, Chicago, USA). Multiple Comparisons on Pearson Chi-square Test was used to analyze 2xC table. A p value <0.05 was deemed as statistically significant.

Results

Distribution of the ASAb in infertility patients

It has been reported that ASAb exhibits adverse impact on appropriate sperm function [21, 22], therefore we determined the ASAb in infertility patients. As presented in **Table 1**, in the female group, it clearly showed that a total 595 out of 1711 in primary infertile women and 445 out of 1100 secondary infertile females were found ASAb positive in serum, on the contrary, only 2.39% positive ASAB was found in healthy female control (P<0.01, both primary and secondary infertile vs healthy control). Same trend was also found in male group, the ASAb posi-

Table 2. The positive rate of EMAb, AOAb and AZP in infertile female

Groups	N	EMAb		AOAb		AZP	
		Positive case (N)	Positive rate (%)	Positive case (N)	Positive rate (%)	Positive case (N)	Positive rate (%)
Primary Infertility	1711	551	32.20[a,b]	629	36.76[a,b]	601	35.13[a,b]
Secondary infertility	1100	118	10.73[a]	34	3.45	38	3.09
Control	627	16	2.55	17	2.23	14	2.71

[a]*P*<0.01 compared with control group; [b]*P*<0.01 compared with secondary infertility group.

Table 3. The positive rate of subtypes of ACA in infertile female

Groups	N	ACA-IgG		ACA-IgM		ACA-IgA	
		Positive case (N)	Positive rate (%)	Positive case (N)	Positive rate (%)	Positive case (N)	Positive rate (%)
Primary infertility	1711	471	27.53[a,b]	577	33.72[a,b]	425	24.84[a,b]
Secondary infertility	1100	37	3.66	15	1.36	17	1.55
Control	627	8	1.28	8	1.28	14	2.23

[a]*P*<0.01 compared with control group; [b]*P*<0.01 compared with secondary infertility group.

tive rate in infertile male population is significantly higher than the normal control (P<0.01, **Table 1**).

Distributions of EMAb, AOAb and AZP in infertile females

We next compared the distributions of EMAb, AOAb and AZP in females, as they all presumably play important roles in immune infertility in women. The serum positive rate of EMAb, AOAb, and AZP in 1711 primary infertile women, 1100 female secondary infertility patients and control group were detected and calculated. As shown in **Table 2**, we observed that the positive rates of EMAb, AOAb and AZP in primary infertility group were all significantly higher than those in control group (EMAb: 32.20% vs. 2.55%; AOAb: 36.76% vs. 3.09%; AZP: 35.13% vs. 2.23%; P<0.01) and patients with secondary infertility (EMAb: 32.41% vs. 10.73%; AOAb: 37.00% vs. 3.09%; AZP: 35.35% vs. 3.45%; P<0.01). Moreover, when we compare the positive rate of EMAb, AOAb and AZP between secondary infertility group with control group, we found that only the serum EMAb positive rate was significantly higher in secondary infertility females than that in control group (10.73% vs. 2.55%, P<0.01). By contrast, no statistical difference was observed with regard to the serum positive rate of AOAb and AZP in secondary infertility females than that in control group (P>0.05).

Distribution of ACA in infertile females

ACA is an autoantibody that been found in SLE, syphilis and idiopathic spontaneous abortion [23-25]. We detected 3 subtypes of ACA in our studies (**Table 3**), which are IgM, IgG and IgA, respectively. Our observations revealed that the population with primary infertility did show significantly higher positive rate of ACA in all three subtypes either compared with control group (IgG: 27.53% vs. 1.28%, IgM: 33.72% vs. 1.28%, IgA: 24.84% vs. 2.23%; P<0.01) or compared with secondary infertility group (IgG: 27.71% vs. 3.66%, IgM: 33.94% vs. 1.36%, IgA: 25.0% vs. 1.55%; P<0.01). By contrast, no significant difference was observed between control group and secondary infertility group in all three subtypes of ACA (P>0.05).

Discussion

In this study, we report a systematic analysis of a series of serum anti-reproductive antibodies in a Chinese population of Tianjin area. We collected 1711 female and 1305 male infertility patients to compare the antisperm antibody in different genders; we show that the ASAb in infertile group is significantly higher than that in healthy group, both in male and female population. Further analysis enrolled 1100 females who suffered from secondary infertility, the results show that the female-associated autoantibodies such as EMAb, AOAb and AZP all have exhibited significant higher positive rate

than both control group and secondary infertility group. Finally, we analyzed the positive rate of ACA antibody, which was previously thought to be involved in idiopathic spontaneous abortion. We discovered that the positive rate of three subtypes of ACA were all significantly higher than secondary infertility and control group. Our analysis, hence, suggested that ASAb, EMAb, AOAb, AZP and ACA might be informative for the diagnosis of primary infertility.

Fertilization consists of a sequence of complicated biological processes that function in a stepwise fashion [26]. The spermatozoon interacts with oocyte in the fallopian tube and binds with the thick layer surround the occyte that is been called zona pellucida and subsequently an acrosomal reaction promotes its fusion with oocyte. Eventually, this process results in the formation of zygote [27]. Then it shifts to the uterus and be embedded in endometrium to achieve the pregnancy. Disturbance of certain steps would consequent in infertility. It has been proposed that immunological factors contribute to approximate 10%-15% of the infertility population in China [28, 29]. Moreover, no standard has been established with regard to the autoantibody test in the infertile or subinfertile population. Therefore, it is of great importance to study the relationship between infertility and these antibodies. ASAb has been shown adversely related to sperm motility and binding activity with oocyte [10, 21, 30]. Under normal conditions, no ASAb can be produced in male and female for the existence of immunosuppression in semen fluid; however, it can be produced in females when the reproductive system experience trauma or infections [31]. What is more, ASAb effectively blocks the acrosome reaction [32], which is the main reason for the failure of natural pregnancy. Although recent meta-analysis showed that no association has been found between ASAb and pregnancy rate after in vitro fertilization (IVF) [33], a number of conclusive studies have been made in terms of the mechanisms of the detrimental effects of ASAb on natural fertility. Our results revealed that women possessed higher ASAb positive rate than that in men in the infertile population of Tianjin area, which might suggest that the immunological impact of women is noticeable. Therefore, we next detected EMAb, AOAb and AZP of women in primary infertility group, secondary infertility group and control group respectively. EMAb is the autoantibody that against the progesterone-dependent protein in the glandular epithelium of endometrium [34]. Like ASAb, it is known that endometriosis stimulates its production thereby inducing local immune-pathogenesis reactions which ultimately results in implantation failure or early abortion. In our study, we found that EMAb positive rate in primary infertile female was significantly higher than control group. In detail, interestingly, we also found a significant difference of EMAb positive rate between secondary infertility group and control group. This suggests that serum EMAb might have strong diagnostic power in infertility. But further separate analysis in terms of EMAb on primary and secondary infertility is still required. Antiovary immune response has the potential to cause disorders of the ovulation process. It is now widely believed that AOAb is associated with oaritis [35]. Given that ovary is the prerequisite place where occytes are developed and matured, AOAb may also contribute greatly to infertility. The source of antiovary antibody is still unclear, but it has been proposed to be induced by the antigens in other organs which resemble those in ovary. Moncayo et.al reported that the positive rates of AOAb in primary and secondary infertility are 22.7% and 37.5% [36], respectively. In our study, the results in primary infertility group are in accordance with their results with a positive rate of 32.1%. However, no significant difference between secondary infertility group and control group were observed, this deviation might result from the different study population of the two studies or the existing selection bias. Nevertheless, our results are in consistent with a previous study which demonstrated that AOAb might be a possible cause of IVF failure [19, 37]. Additionally, we observed that in primary infertility group, the positive rate of AZP, a fore mentioned autoantibody that involved in infertility and prohibiting the acrosome reaction was significantly higher than that in control group, whereas no difference was found in secondary infertility group compared with control. Our results suggest that AZP and AOAb are sensitive in the diagnosis of primary infertility.

Finally, we also compared the ACA positive rate in each groups, for that ACA is an important auto-immunological antibody that involved in syphilis, SLE and idiopathic spontaneous abortion, especially. It has been shown competitively binding to the phospholipid receptor, which

results in thrombogenesis in placenta and ultimately abortion. The positive rate of ACA-IgG, ACA-IgM and ACA-IgA subtypes in our study population are 27.71%, 353.94% and 25.0% in primary infertility group, respectively. Importantly, they all showed significant difference when compared with control or secondary infertility group. Therefore, we conclude that ACA also shows clinical significance in the diagnosis of primary infertility and it should be taken into account in its diagnosis and treatment.

It should be noted that our study just provided pilot observational data of the anti-reproductive antibodies in infertile female, thus several limitations might confound the results. For instance, our study is only observational and retrospective rather than prospective; no causal-link has been established. Moreover, selection bias may exist in our study, which calls for a cautious analysis in the future.

In summary, the present study has shown that a batch of reproductive system related autoantibodies are found to have higher detection ratio in primary infertility female, which implicated the importance of auto-immunological factors in primary infertility and may give a hint in its diagnosis and treatment. However, although there is very high auto-immunological factors in the current study, we think there are still lots of other factors to influence the infertility, we cannot overestimate the contribution of auto-immunological factors in infertility.

Disclosure of conflict of interest

None.

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