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Involvement of reproductive system related autoantibodies in Chinese patients with infertility: A reproductive immunology study.

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Abstract

Objective: To evaluate the role of auto-antibodies in Chinese patients with primary and secondary infertility in routine clinical practice.

Methods: We reviewed the medical records of infertile Chinese patients of either gender (≥ 18 year old) who were failed to conceive after 12 months of regular sexual intercourse without the use of contraception, and visited at department of Gynaecology and Obstetrics, Renmin Hospital of Wuhan University, China between January 2004 to December 2014, and undergone auto-antibody estimation along with other routine antenatal screening tests. Immunological assay was performed using dot immuno-gold filtration assay. Positive case was compared among patients with primary and secondary infertility.

Results: Our chart review study showed that the positive case of Anti-Sperm Antibody (ASAb) was significantly higher in female with primary than male ($p<0.05$). In female patients with primary infertility, all other auto-antibodies (anti-Endometrium Antibody (EMAb), Anti-Ovary Antibody (AOAb), Anti-Zona Pellucida Antibody (AZP)) and Anti-Cardiolipin Antibody (ACA) were significantly higher compared to patients with secondary infertility ($p<0.05$). Moreover, positive case of all auto-antibodies was significantly higher among female with primary fertility than secondary fertility and control groups.

Conclusion: Our study results suggested the involvement of auto-antibodies related to reproduction system in development of primary infertility. Auto-antibodies related to reproduction were significantly higher in patients with primary infertility, this implicated involvement of auto-immunological factors in development of infertility.

Keywords: Auto-antibodies, Primary infertility, Secondary infertility, Anti-sperm antibody, Anti-endometrium antibody, Anti-ovary antibody, Anti-zona pellucida antibody.

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Introduction

Infertility refers to the biological inability of an individual to contribute to conception. In many countries 'infertility' refers to a couple that has failed to conceive after 12 months of regular sexual intercourse without the use of contraception. The incidences of infertility were increases to 4% throughout the world after '80s [1]. Among Chinese population, approximately 10-15% of married men and women are suffering from infertility. Infertility badly impact the quality of life of married men and women who are unable to contribute to conception, it also add financial burden to their family by consulting gynaecologist and undergo numerous laboratory tests [2].

In a recent study conducted in China revealed that the infertility rate among married couples was observed to be increased by 12.5% (earlier two decade ago, this was only 3%) [3]. Some experts believe that the reason for the higher infertility rates in females and males is mainly as a result of unhealthy lifestyle. Others suspect that this is due to the environmental conditions which may also play a major role. The other factors associated with this etiology are the DNA damage, endocrine system disorders, genetic factors and environmental factors [4]. It is already evident that, sperm of infertile men contain more DNA damage than fertile men and this may have a negative effect on probable fertility chances of these patients [5].

The definitive cause of infertility is still ambiguous and to be determined. Effective treatment to infertility is possible if the cause of infertility is known. The recent studies highlighted the influence of immune system on women fertility and this led to the urgency in the reconnaissance of reproductive immunology [6]. It has been elucidated that the presence of anti-reproductive antibodies in serum of men and women is one amongst the major cause of immune infertility. It has been reported that presence of key antibodies such as anti-sperm, anti-endometrium, anti-ovary, anti-zona pellucida and anti-cardiolipin antibody affect fertilization and implantation process which results in infertility. Few *in-vitro* studies suggested the possible involvement of few auto-antibodies in failure of fertilization and implantation process. In another studies, presence of Serum Anti-Endometrium (AEM), Anti-Ovary (AO), Anti-Zona Pellucida (AZP) and Anti-Cardiolipin (AC) antibody was observed among infertile patients [7-10]. Since several lines of clinical evidences suggest the possible role of auto-antibodies in infertility, however there was no real world evidences evaluating infertile patients with existence of auto-antibodies [11-13]. Therefore, we designed this retrospective study to identify the presence of auto-antibodies in large number of Chinese patients in routine clinical practice.

In the present study, we reviewed the medical records of infertile Chinese patients of either gender (≥ 18 year) visited at department of Gynaecology and Obstetrics, Renmin Hospital of Wuhan University, China between January 2004 to December 2014 due to complaint of infertility and undergone auto-antibody estimation. This was the first and largest retrospective analysis for the presence of auto-anti-bodies in infertile patients in routine clinical practice.

Subjects and Method

We reviewed the medical records of 6500 successive Chinese patients of either gender (≥ 18 year) visited at department of Gynaecology and Obstetrics, Renmin Hospital of Wuhan University, China between January 2004 to December 2014 due to complaint of infertility. Computerized database of Renmin Hospital of Wuhan University, China to collect required data was used to fulfil the objective of our study. All the patients whose medical records were reviewed were suffering from either primary or secondary infertility. As per the protocol of our hospital, all the patients who visited for consultation of infertility were undergo laboratory and physical examination including urological and gynaecologic assessments to rule out the possibility of any diseases which causes infertility. In women with child bearing age, fallopian tube radiography and patent test was performed to confirm normal patency in their fallopian tube. All female patients were subjected to confirm the normal functioning of ovulation. Sperm count, sperm motility and viability of each male patient was checked and recorded. Presence of systemic lupus erythematosus or any other rheumatic diseases was evaluated during first visit. Also patients who were lupus anticoagulant positive were recorded.

For infertility group, we have excluded the medical records of patients who were lupus anticoagulant positive. We have omitted the data of individuals with presence of systemic lupus erythematosus or any other rheumatic diseases. Also medical records of patients with active disease of reproduction system which causes infertility were also excluded. Medical records with any significant abnormality in fallopian tube were also excluded. The subjects who had normal laboratory results were considered in control group. Institutional ethics committee approval was received. Since, this was a retrospective, observational study, and patients whose medical records reviewed were not contacted, or are named in the study, thus the requirement for obtaining formal informed consent was waived by ethics committee. As a routine procedure of our hospital, blood sample was collected from each patient for autoantibodies assessment from patients who had diagnosed with primary and secondary infertility. Immunological assay was performed using dot immuno-gold filtration assay. All the laboratory measurement for detection of antibodies was performed according to manufacturer's instruction. In dot immuno-gold filtration assay, red color dots indicate positive results; dots with no color were counted as negative. In our study, positive case and positive rate of each measured antibodies was measured. Positive case and positive rate was compared among patients with primary and secondary infertility.

Quantitative variable was presented as mean ± standard deviation, and data were compared using parametric/non-parametric statistical test based number of comparison group and distribution of data, using 2 sided statistical tests. Normality test (Kolmogorov-Smirnov test or Shapiro-Wilks test) will be used to check the distribution of data of quantitative data. A categorical variable was presented as absolute number and/or percentage of subjects in each category, and were compared using Chi-square or fisher exact test, using 2 sided statistical tests. In all cases, a $P<0.05$ was considered to be statistically significant among comparison groups. Data from each patient was coded and analysed using Graph Pad Prism software for statistical analysis (version 6.0).

Results

Medical records of total 6500 individuals with average age of 32.67 (2.8) were reviewed. Of these, 5,600 patients' data were met criteria for inclusion in analysis. A total of 3900 patients had primary infertility, while 1700 female patients had secondary infertility. In primary infertility group, 1600 patients were male and 2300 patients were female.

Role of anti-sperm antibody (ASAb) in infertility

We have analysed the presence of ASAb in infertility patients since this antibody is known to impair the function of sperm [14-18]. As indicated in Table 1, positive cases of ASAb were significantly higher among female patients with primary infertility than secondary fertility. When comparison was made between male with primary infertility and female patients with primary fertility, the percentage of positive cases of ASAb was

significantly higher in female patients with primary fertility than male patients with primary fertility.

Role of anti-zona pellucida antibody (AZP), anti-endometrium antibody (EMAb), anti-ovary antibody (AOAb) in female patients with infertility

We found that the positive case of EMAb, AOAb and AZP were significantly high in female patients of primary fertility than patients with secondary fertility. We have analysed the presence of EMAb, AOAb and AZP in female patients since these antibodies are involved in immune fertility in women with child bearing age [19,20]. Summary of positive cases of EMAb, AOAb and AZP are described in Table 2. When comparison was made between patients with primary and secondary infertility patients, the percentage of positive cases of EMAb, AOAb and AZP were significantly higher in female patients with primary fertility than secondary fertility. When comparison was made between control groups with primary and secondary infertility patients, we found that the number of positive cases of EMAb, AOAb and AZP were significantly lower in control group when compared with patients with primary fertility than secondary fertility. Increased level of EMAb, AOAb and AZP was found to cause primary fertility in women of child bearing age.

Role of anticardiolipin antibody (ACA) in female patients with infertility

We have analysed the presence of ACA in female patients since this antibody is involved in syphilis and idiopathic spontaneous abortion in women with child bearing age. We found three subtype of ACA namely Immunoglobulin G, M and A (Ig G, Ig M and Ig A). We found that the positive case of ACA of all sub type (Ig G, Ig M and Ig A) were significantly high in female patients of primary fertility than secondary fertility. Summary of positive cases of ACA of all sub type (Ig G, Ig M and Ig A) are described in Table 3. When comparison was made between patients with primary and secondary infertility patients, the percentage of positive cases of ACA of all sub type (Ig G, Ig M and Ig A) were significantly higher in female patients with primary fertility than secondary fertility. When comparison was made between Control groups with primary and secondary infertility patients, we found that the number of positive cases of ACA of all sub type (Ig G, Ig M and Ig A) were significantly lower in control group when compared with any of infertility group. Increased level of ACA of all sub type (Ig G, Ig M and Ig A) was found to cause infertility in women of child bearing age.

Table 1. *Number of patients with positive results of ASAb among patients with infertility.*

Gender	Group	Number of patients with positive ASAb case
Men (N=1600)	Primary infertility*	1267
Men (N=600)	Healthy group	2
Female (N=2300)	Primary infertility*	1364
Female (N=1700)	Secondary infertility*	896
Female (N=600)	Healthy group	5

Values are expressed as absolute number of patients in each category. *p<0.001 for comparison between primary and secondary infertility. *p<0.001 for comparison between primary and secondary infertility *vs.* Healthy/control group.

Table 2. *Number of patients with positive results of EMAb, AOAb and AZP in female patients with infertility.*

Type of fertility	Number of patients with positive case	Percentage of patients with positive case	P-value
Anti-Endometrium Antibody (EMAb)			
Primary infertility (N=3900)	1989	51%	<0.05
Secondary infertility (N=1700)	821	48%	
Healthy group (N=600)	12	2%	
Anti-Ovary Antibody (AOAb)			
Primary infertility (N=3900)	1432	37%	<0.05
Secondary infertility (N=1700)	512	30%	
Healthy group (N=600)	14	2.33%	
Anti-Zona Pellucida Antibody (AZP)			
Primary infertility (N=3900)	1897	49%	<0.05
Secondary infertility (N=1700)	712	42%	
Healthy group (N=600)	12	2%	

Values are expressed as absolute number and percentage of patients in each category.

Table 3. *Number of patients with positive results of ACA among patients with infertility.*

Type of fertility	Number of patients with positive case	Percentage of patients with positive case	P-value
ACA Ig G			
Primary infertility (N=3900)	1892	49%	<0.001
Secondary infertility (N=1700)	721	42%	
Healthy group (N=600)	16	3%	
ACA Ig M			
Primary infertility (N=3900)	1539	39%	<0.001
Secondary infertility (N=1700)	486	29%	
Healthy group (N=600)	13	2%	
ACA Ig A			
Primary infertility (N=3900)	1765	45%	<0.001
Secondary infertility (N=1700)	712	42%	
Healthy group (N=600)	15	2%	

Values are expressed as absolute and % of number of patients in each category.

Discussion

To the best of our knowledge, this was the first largest retrospective chart review study assessing the involvement of auto-antibodies among Chinese infertile patients. We reviewed the medical records of infertile Chinese patients of either gender (≥ 18 year old) who were failed to conceive after 12 months of regular sexual intercourse without the use of contraception, and visited our hospital (Department of Gynaecology and Obstetrics, Renmin Hospital of Wuhan University, China) between January 2004 to December 2014, and undergone auto-antibody estimation along with other routine antenatal screening tests. Our chart review study showed that the positive cases of ASAb are higher in patients of primary fertility than the patients with secondary fertility. Anti-sperm antibody was involved in impairment of sperm [21]. When we compared ASAb test report with the other healthy subjects having all test normal, we found that ASAb test was negative among subject who is having normal range of all laboratory tests. Similar trend was observed when comparison was made for EMAb, AOAb and AZP. Study results showed incidence of positive case of EMAb, AOAb and AZP were significantly high in female with primary fertility than secondary fertility. Our study results suggested the increased level of EMAb, AOAb and AZP was found to cause infertility in women of child bearing age. We also analysed the presence of ACA in female patients since these antibodies are involved in syphilis and idiopathic spontaneous abortion in women with child bearing age. We found three subtype of ACA namely Immunoglobulin G, M and A (Ig G, Ig M and Ig A). Study showed that the positive case of ACA of all sub type (Ig G, Ig M and Ig A) were significantly high in patients of primary fertility than patients with secondary fertility. On comparison between control groups *vs.* primary and secondary infertility patients, we found that the number of positive cases of ACA of all sub type (Ig G, Ig M and Ig A) were significantly lower in control group when compared with any of infertility group. Increased level of ACA of all sub type (Ig G, Ig M and Ig A) was found to cause infertility in women of child bearing age. Anti-Sperm Antibody (ASAb) significantly impairs the sperm motility and binding capacity of sperm with oocyte results in no formation of zygote lead to infertility [10,21-23]. Anti-sperm antibody is usually not present among healthy married couple, presence to ASAb in serum of either partner prevent the existence of natural pregnancy. Anti-sperm antibody formed in male or female due to trauma in reproduction system. In addition, Anti-sperm antibody inhibits acrosome reaction which is important step in inducing natural pregnancy, this leads to failure of natural pregnancy [21,22]. However, results of recent systemic reviews suggested that there is no relation between anti-sperm antibody and pregnancy as observed in *in-vitro* fertilization [23]. We found involvement of ASAb among the infertile patients and the incidences of positive case of ASAb were significantly higher in female patients with primary fertility as compared to male who had primary fertility.

We also recorded the positive cases of anti-endometrium antibody in serum sample of female patients. Anti-endometrium antibody is a type of auto-antibodies for progesterone based protein in endometrium [22]. Similar to anti-sperm antibody, stimulation of this antibody results in occurrence of local immunogenic reaction which leads to impairment of implantation process or early abortion.

Antiovary immune was found to cause disturbance in ovulation process, and assumed that AOAb directly linked with oaritis [23]. Since ovary is key location for production and maturation of oocytes, thus AOAb could be accountable for infertility.

Anti-Cardiolipin Antibody (ACA) is essential autoantibodies that are mainly associated with etiology of abortion. ACA is the key auto-immunological antibody mainly involved in development of syphilis, systemic lupus erythematosus and idiopathic spontaneous abortion, especially. It has reported that ACA competitively bind to the phospholipid receptor in placenta, results in thrombogenesis and lead to abortion. In our study, we found that the positive case of ACA of all sub type (Ig G, Ig M and Ig A) were significantly high in patients of primary fertility than patients with secondary fertility. We, therefore, conclude that ACA plays very important role in the diagnosis of primary infertility and it should be taken into account in its diagnosis and treatment.

Conclusion

Our study results suggested the involvement of auto-antibodies related to reproduction system in development of infertility. Auto-antibodies related to reproduction were significantly higher in patients with primary infertility, this implicated involvement of auto-immunological factors in development of primary infertility. In present study, we focused on auto-immunological factors; however, there are several other factors which contribute in cause of infertility. We, therefore, could not overemphasize the role of auto-immunological factors in infertility.

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