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RESEARCH ARTICLE

A SURVEY OF ANTISPERM ANTIBODIES AND ASSOCIATION WITH INFERTILITY AMONGST MEN IN BENIN CITY, NIGERIA

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ABSTRACT

Immunological infertility induced by antisperm antibodies (ASA) may interfere with fertilization or decrease the chances of pregnancy occurring. This study was conceived to determine the prevalence of ASA in the serum in infertile men and women and their relationship to semen quality. The study involved 209 infertile men, 212 infertile women and 114 fertile men and 118 fertile women as control. Sera were examined by the indirect spermmixed antiglobulin test (MAR)test and micro – titer tray agglutination test (TAT). Semen parameters were determined by standard techniques and ASA in semen was evaluated with direct mixed antiglobulin test. ASA were detected at clinically significant titres (≥32) in the sera of 5/209 (2.4%) and 23/212 (10.8%) infertile men and women respectively. The concentration and progressive motility of spermatozoa from infertile men with ASA declined significantly ($P < 0.0001$) with altered distribution of white blood cells populations. It is concluded that ASA when present in the serum may adversely affect the quality of semen vis-a-vis fertilization and are associated with asthenosoozspermia and or oligospermia and altered distribution of white cell populations which represent important markers of immunologically induced infertility.

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INTRODUCTION

The correlation of antisperm antibodies (ASA) with some instances of unexplained infertility has been reported to have implicating role for these antibodies in blocking fertilization (Diekman*et al.,* 2000). Immunologically induced infertility has been reported to account for 3% of male infertility (Rowe *et al.,* 2000; Ni *et al.,* 2000, Marzena*et al.,* 2003) and 4.6% of the female partners of infertility in marriages (Witken and Chaudhry, 1989). ASA have been indicated to affect fertility in several ways which result in poor prognosis for normal treatment modalities for infertility cases. (Shibahara and Konyama, 2013). Studies have also shown that pregnancy rates were significantly reduced or lower when greater than 30% of spermatozoa were covered with surface ASA of the 1gG or 1gA class of immunoglobulins (Eggert – Kruse *et al.,* 1991; Vazquez – Levin *et al.,* 1991). These antibodies interfere with various stages of the fertilization process (Rajah *et al.,* 1993; Restrepo and CardonaMaya, 2013).

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ASA titre of ≥ 32 in the microtitre test has been considered clinically significant. (Krapez*et al.,* 1988). It has also been reported that titres≥ 256 is associated with conception rate of zero (Meinertz*et al.,* 1990). The disruption of the blood-testes barrier has been implicated in the induction of ASA production (Comhaire*et al.,* 1991). Sperm antigens then initiate infertile men to stimulate heightened immune responses to sperm antigens and altered distribution of white cell populations in both partners (Mathur*et al.,* 1990). Immunological infertility is given little or no attention in the management of infertility cases in most treatment centres in Nigeria, the current study was therefore designed to determine prevalence of ASA and the associated indicators of immunological infertility in Edo and Delta States, Nigeria.

MATERIALS AND METHODS

Study population: This consisted of consecutive male and female patients on fertility work-up attending seven medical centres in Edo and Delta States. The control consisted of previously screened donors and fertile men that gave informed consent. Each male subject was asked to abstain from passing

semen for 72h, wash hands and genitals with mild toilet soap and dry skin with a sterile disposable towel provided. Thereafter the subject was asked to collect semen by masturbation into a sterile bottle supplied and deliver to the laboratory within 1h of collection.

Sample collection and examination: A sample of blood (4ml) was collected from each male and female subject with 2.0ml dispensed unto 1mg/mL ethylenediamine-tetra- hydrochloric acid (EDTA) anticoagulant bottle, well-mixed and processed within 2h the remaining 2.0ml was dispensed unto a plain bottle and kept at 8^{o}C in the refrigerator for the clot to retract for 2h. The clot was loosened with an applicator stick and spun at 300 G for 3min. The resulting serum was separated unto a fresh plain container, and stored–20^{o}C until assay time. Each sample of semen was examined for spermatozoa concentration and motility characteristics using standard techniques (WHO, 2010).

Antiperm antibody detection mixed antiglobulin reaction test (MAR): Each sample of semen was well–mixed and 3.0µL was removed and placed on a clean microscope slide. To this was added 3.0µl of washed group O red blood cells sensitized with goat anti-human IgG by stirring with the pipette tip. The suspension was covered with a cover-slip and allowed to stand in a wet chamber for 3min before examination with x20 and x40 objective of the phase contrast microscope and again after 10min. Spermatozoa with ASA on their surface were seen as motile sperm cells with red blood cells attached to them (Hendry *et al.,* 1982). Sera were tested with the indirect sperm MAR kit (Beerman, Belgium) and positive samples titres were determined by micro titre tray agglutination test (TAT).

Tray Agglutination Test (TAT): Sera with positive MAR tests from men with direct positive semen MAR and indirect MAR test from women subjects were diluted in 5ul volumes in micro-titre plates (1:2; 1:4; 1:8; … 1:1024). A known MAR negative semen with total motility $\geq$ 60% and spermatozoa concentration $\geq$40 x 10^{6}/mL was diluted to give 5x10^{6}/mL in phosphate buffered saline (PBS) to which was added 20% bovine serum albumin, BSA (Sigma-Aldrich Biochemicals and Reagents, USA). To each micro-well was added 5uL of diluted semen. The preparation was covered with a layer of paraffin oil and incubated at 37^{o}Cin an incubator. The assay was read after 1h with x10 and x20 microscope objective. The highest dilution of serum agglutinating the sperm cells was recorded as the titre.

Total and differential white blood cells (WBCs) count: The total and differential WBCs was determined from each sample using flow cytometer (ERMA-PCE 210N, Japan). The instrument differentiates cells from whole blood into lymphocytes, monocytes and granulocytes. The assayed was achieved by switching on the equipment while the sample in EDTA was mixed in a rotating mixer for 5min, the equipment was flushed with buffer and control sample was applied. This was followed with the test sample and results displayed were recorded (Rodak, 1995).

CD4 count: This was carried out with the aid of Cyflow–SLS flow-cytometer (Partec, Germany) which features a complete automated system. Prior to the assay, the equipment was flushed with a buffer and calibrated with a blank. A control sample of known CD4 count was applied. Thereafter, the test sample was applied and the result displayed digitally was recorded (McClatchey, 1989).

Data was analyzed using the Chi (X^2) and degree of confidence was set at 95% ($P < 0.05$).

RESULTS

The detection rate of antisperm antibodies in the serum of the study subjects is presented in Table 1. Antisperm antibodies at clinically significant titres were detected in 5/209 (2.4%) of infertile men. ASA were not detected at this level from fertile men. ASA were recorded at titres lower than 32 in 2.6% of fertile men compared to 11.0% of infertile women. Clinically significant titres were found in 23/212 (10.8%) of infertile women which is almost four-folds that recorded in infertile men. ASA were detected at titres lower than 32 in 4.2% of the fertile men. The comparison of spermatozoa concentration and progressive motility in serum of study subjects with ASA is displayed in Table 2. The concentration of spermatozoa was significantly higher ($P<0.0001$) in semen without ASA – 38x 10^{6}/mL compared to 13.8x10^{6}/mL in semen with clinically significant titres. In semen with ASA titre levels lower than 32, sperm counts were also significantly lower than in samples from infertile men without ASA. Progressive motility declined significantly ($P< 0.0001$) from 32.5% to 7.1% with ASA titre of 32 or higher. Motility ratio also declined in semen with ASA titre lower than 32. Progressive motility of spermatozoa at different ASA titres in sera of subjects is presented in Table 3. The mean progressive motility of spermatozoa from in fertile men without ASA was 37.3% compared to 58.3% in infertile without ASA. Motility was only 8.9% in semen of infertile men with ASA tire lower than 32 in comparison to 58.3% in fertile men. ASA were not detected at a titre of 32 or higher in fertile men. White blood cells populations from infertile and fertile men and women is displayed in Table 4. The total WBCs counts were significantly higher ($P< 0.0001$) in both infertile men and women. The sub-population of neutrophils and lymphocytes were significantly increased ($P< 0.0001$) in infertile subjects. The CD4 counts in fertile women also significantly increased ($P< 0.0001$). The increase in CD4 levels from infertile men were however not significant different ($P = 0.0528$). The populations of mixed granulocytes other than neutrophils did not vary significantly ($P>0.05$) in both infertile and fertile subjects.

Table 1. Detection levels of ASA in serum of subjects

Subjects group	Frequency of ASA titre (%)	
	$\leq$16	$\geq$ 32
Men; Infertile (n = 209)	23(11.0)	5(2.4)
Fertile (n = 114)	3(2.6)	0(0.0)
Women; Infertile (n = 212)	38(17.9)	23(10.8)
Fertile (n = 118)	5(4.2)	0(0.0)

Table 2. Comparison of spermatozoa concentration and progressive motility in the presence of ASA in the serum of infertile men

ASA Titre	Spermatozoa concentration (x 10^{6}/mL)			Progressive Motility (%)	
	No. (%)	Range	Mean	Range	Mean
0	181 (86.6)	0.9 – 124	38.4	5 – 75	32.5
$\leq$ 16	26(11.0)	0.0 – 61.5	17.8	0.0 – 31.3	18.6
$\geq$ 32	5(2.4)	0.0 – 46.0	13.8	0.0 – 25.0	7.1

Table 3. Mean progressive motility of spermatozoa at different ASA titre in serum of subjects

Subject group	ASA Titre					
	0		< 16		> 32	
	Motility	No. (%)	Motility	No. (%)	Motility	No. (%)
Infertile (n = 209)	37.3	162 (77.5)	8.9	33(15.8)	4.0	5(2.4)
Fertile (n = 114)	58.3	106 (93.0)	46.9	8(7.0)	-	0(0.0)

Table 4. Total white blood cell populations and differential counts

Blood cell parameter	Men		Women	
	Infertile	Fertile	Infertile	Fertile
Total WBCs/μL	4,580	3,613.5	4,506.3	6,168.9
CD4/μL	906.1	823.3	1,002.6	908.9
Neutrophils (%)	41.2	56.2	41.0	64.5
Lymphocytes (%)	54.4	36.2	51.7	26.8
Mixed granulocytes (%)	4.9	5.1	7.8	8.0

DISCUSSION

Antisperm antibodies (ASA) were detected in 5/209 (2.4%) of infertile men and 23/212 (10.8%) of infertile women at clinically significant titres. Other studies have reported 2.0 – 7.3% in the serum of infertile couples (Ni *et al.,* 2000; Rowe *et al.,* 2000; Marzena*et al.,* 2003; Hadinedoushan and Gharfourzadeh, 2007). The prevalence of ASA in infertile couples in Nigeria is also close to the ranges in other parts of the world. However, a previous study in Nigeria found 44% of infertile men sera positive for ASA (Ekwere, 1995). This high prevalence didn't state the titre considered which could have been responsible for the higher rate. A higher rate recorded in infertile women from this study may have been exacerbated by the poor health infrastructures available to the study population coupled with a high prevalence of genital tract infections. ASA were all lower than 32 in the fertile men and women groups. This may infer that ASA titres lower than 32 may not interfere with fertility as has been reported in other studies (Hargreave and Hjort, 2009). The mean spermatozoa concentration in infertile men without ASA was significantly higher (P<0.0001) in comparison to those from infertile men with ASA lower than 32. This is an indication that, ASA even at low titres may exert adverse effects on spermatozoa concentration either by inhibiting sperm cells production or maturation processes resulting in reduced spermatozoa concentration (oligosperrnia). The progressive motility of spermatozoa from men with clinically significant titres of ASA declined significantly (P<0.0001) when viewed against samples from infertile subjects without ASA (Table 2).

This is further suggestion that ASA have immobilizing effect on spermatozoa leading to weakened, reduced mobility and in some cases complete immobility (asthenozoospermia). This inevitably will interfere with the essential mobility requirement for sperm cells to accomplish fertilization of the ovum spontaneously *in–vivo*. This observation is similar to the conclusion from studies elsewhere (Restrepo and Cardona-Maya, 2013). In addition, the coating of the sperm cell with ASA usually leads to the modification of sperm cells binding site to the ovum. This ultimately results in reduced affinity or loss of the ability to bind to the ovum and the consequence of fertilization failure. Semen samples from infertile men with ASA titres lower than 32 also had lower progressive motility. Progressive motility in semen from fertile men with ASA titres which were all lower than 32 were not significantly different (P > 0.05) from fertile subjects without ASA. This may be why reports have indicated that ASA at low titres do not interfere with fertilization. (Meinertz*et al.,* 1990; Krapez*et al.,* 1998).

This study is a further pointer that ASA may be a secondary factor resulting from an underlying modulator such as bacterial infection that may wholly or in part be responsible for interfering with fertility. The total white blood cells populations in infertile men and women were significantly increased (P<0.0001). The sub–populations of neutrophils and lymphocytes were also significantly elevated (P < 0.0001) in infertile subjects as has been reported from similar studies (Marthur*et al.,* 1990). This may also imply that a degree of leukocytosis or leukocytospermia is always associated with male infertility when ASA is present. The consequence of the presence of leukocytes in semen which are the precursors of reactive oxygen species (ROS) then initiate the damage of sperm cells and function through the induction of oxidative stress (OS) on sperm cell. This is consistent observations from other investigations (Omu*et al.,* 1999; Benedetti *et al.,* 2012). The population of CD4 cells in infertile women were significantly heightened (P<0.0001) as other studies have reported (Liu, *et al.,* 1993). The CD4 cells population in infertile men also increased but the increase was not significant (P = 0.0528) in comparison to the fertile men. The possible explanation may not be unrelated to the ability of men generally to get better adapted to stressful conditions than women. This study has revealed that asthenozoospermia or oligoasthenozoospermia and altered distribution of white blood cells' populations are important makers of immunologically induced infertility in the study population. It is therefore expedient that clinical laboratories operating in Nigeria should make more deliberate efforts at detecting and quantitating ASA.

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