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PREVALENCE OF CHLAMYDIA AND LEVELS OF SEMINAL ANTISPERM ANTIBODY AND OXIDATIVE STRESS MARKERS OF INFERTILE MEN IN CALABAR, SOUTHERN NIGERIA

Iya Eze Bassey[1], Idongesit Kokoabasi Paul Isong[1], Uwem Okon Akpan[1*], Bebe Emini Eleng[1], Euphoria Chimuanya Akwiwu[2], Stephen Godwin Agbara[1], Edoise Momodu Isiwele[3], Alphonsus Ekpe Udoh[1]

[1]Department of Clinical Chemistry and Immunology, Faculty of Medical Laboratory Science, University of Calabar, Calabar, Nigeria.
[2]Department of Haematology and Blood Transfusion Science, Faculty of Medical Laboratory Science, University of Calabar, Calabar, Nigeria.
[3]Department of Urology, University of Calabar Teaching Hospital, Calabar, Nigeria.

***Corresponding Author:** Mr Uwem Okon Akpan, Department of Clinical Chemistry and Immunology, Faculty of Medical Laboratory Science, University of Calabar, PMB 1115, Calabar, Nigeria
Email: akpauwem11@gmail.com **Tel. No.:** +2347031632967

HIGHLIGHTS

- Male infertility occurs globally especially in Africa and has a multi-factorial etiology but women bear most of the brunt of the social stigma and burden.
- This study assessed prevalence of chlamydia and levels of seminal anti-sperm antibody (ASA), total antioxidant capacity (TAC), catalase and vitamin C of infertile men in Calabar, Southern Nigeria.
- This was a case-control study involving 35 oligospermic, 30 azoospermic men, and 30 normospermic fertile men. Seminal chlamydial IgG antibody and ASA were done by Enzyme-linked immunosorbent assay.The prevalence of chlamydia and ASA in infertile men in this study was 4.4% and 0% respectively, seminal TAC, catalase, and Vitamin C were lower and ASA higher in infertile men than fertile men.

Abstract

Background: Male infertility occurs globally especially in Africa and has a multifactorial aetiology but women bear most of the brunt of the social stigma and burden.

Objective:
This study assessed prevalence of chlamydia and levels of seminal antisperm antibody (ASA), total antioxidant capacity (TAC), catalase and vitamin C of infertile men in Calabar, Southern Nigeria.

Methodology:
This was a case-control study involving 35 oligospermic, 30 azoospermic men, and 30 normospermic fertile men. Seminal chlamydial IgG antibody and ASA were done by Enzyme-linked immunosorbent assay. TAC, Vitamin C, and catalase activity were determined by colorimetry and semen analysis by WHO method. Statistical analysis was done using SPSS version 18.0 and data obtained was analyzed by the ANOVA, LSD Post hoc analysis, and Pearson's correlation, and the level of significance was set at $P < 0.05$.

Results:
The oligospermic and azoospermic men had significantly lower ($p = 0.0001$) sperm count, sperm concentration, percentage sperm motility, catalase, TAC and significantly higher ($p = 0.002$) ASA than controls. Only the oligospermic men

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had significantly lower (p = 0.017) Vitamin C levels than the controls. They also had significantly higher catalase (p = 0.003) compared to the azoospermic men. None were positive for ASA and 4.4% of the infertile men were positive for chlamydia IgG antibody. Vitamin C correlated significantly and negatively with ASA (r= -0.388, p =0.009).

Conclusion: The prevalence of chlamydia and ASA in infertile men in this study was 4.4% and 0% respectively, seminal TAC, catalase, and Vitamin C were lower and ASA higher in infertile men than fertile men.
Keywords: Male infertility, chlamydia, antisperm antibody, oxidative stress, catalase, total antioxidant capacity

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1.0 Introduction

The World Health Organization (WHO) defines infertility as a couple's inability to conceive within one year of exposure to regular unprotected intercourse.[1] Over 60 to 80 million couples worldwide experience infertility at some point in their reproductive lives.[2] Africa is believed to share by far the largest burden of infertility, presumably due to poor research and healthcare delivery systems.[3] It is estimated that an average about one in every ten couples are infertile in Africa, with some countries reporting prevalence as high as thirty–two percent.[4]

Male infertility has been implicated in about half of the infertility cases, but the women bear most of the brunt of the social stigma and burden as shown by reports indicating that in Nigeria, the most common reason for gynaecological consultation is infertility.[5] Multicenter studies show that 25-30% of infertility can be attributed to problems in both partners; 25-30 % to male factors, 30-35% to female factors, and for the rest there are no defined causes.[6] The causes of male infertility include but are not limited to genetic conditions, infections, hormonal deficiency, nutritional, and immunological conditions, and idiopathic male infertility or infertility of unknown origin.[7] There is growing evidence of a link between oxidative stress (OS) and male infertility. OS occurs when Reactive Oxygen Species (ROS) production overwhelms the antioxidant defense system,[8] resulting in serious cellular damage if OS is prolonged and/or massive.[9] ROS are by-products of normal sperm processes and leucocyte activity which are necessary for hyperactivation and capacitation of spermatozoa and fighting bacteria. Semen possesses numerous antioxidant systems including enzymatic antioxidants (such as Catalase and superoxide dismuthase) as well as non-enzymatic antioxidants such as Vitamin C and E.[10] In particular, ascorbic acid has been reported to be involved in the maintenance of sperm cell plasma membrane integrity. The total activity of these antioxidants can be assessed by measuring total antioxidant capacity which also serves as an indirect measure of oxidative stress since low levels indicate high levels of oxidative stress.[11]

Infectious diseases have been linked with abnormal sperm parameters and can cause spermatogenic dysfunction. However, chlamydial infection in men, unlike in women, has been understudied, notwithstanding their similar infection rates. It is possible that Chlamydia might contribute to idiopathic male infertility.[12] Nwankwo and Sadiq[13] reported a seroprevalence rate of 10.7% in Kano

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(Northwestern Nigeria) and Enwuru and Umeh [14] reported a 4.7%. in Owerri (South eastern Nigeria). Antisperm antibodies (ASA) are antibodies against sperm which can be sperm-bound or located in seminal plasma or serum. Clinically, they are detected in 3-12% of infertility men. They are formed when there is a breach of the blood-testis barrier, an active immuno-suppression defect or overwhelming sperm antigen inoculations. Their effects include inhibiting sperm progression through the genital tract of the female by immobilization or agglutination thereby reducing the motility and fertilizing capacity of the sperm.[7] Some Nigerian studies have reported prevalence as low as 2.4% in Benin city[15] and as high as 26.67% in Port Harcourt.[16] There is no data for our region as the only studies available were on women and this great variability in ASA prevalence may be an indicator of regional differences in ASA prevalence in Nigeria. There is therefore a need to know if ASA is a key factor of male unexplained infertility or not in our region. This study therefore assessed prevalence of chlamydia and levels of seminal antisperm antibody, total antioxidant capacity, catalase and vitamin C of infertile men in Calabar, Southern Nigeria.

2.0 Materials and methods

Selection and description of participants

A case-control study design was used for this study. A total of 80 participants were consecutively recruited for the study. They were aged between 25 to 50 years. They consisted of Forty-five (45) known infertile men (infertile for at least a year) of Nigerian origin attending the Urological Clinic of the University of Calabar Teaching Hospital, Calabar, Cross River state who were recruited as test subjects. Thirty five (35) apparently healthy fertile men with who had had at least a child in the last two years were also consecutively recruited as controls from Calabar metropolis in Cross River state. The subjects were classified after semen analysis as normospermic (n = 35), oligospermic (n = 35) and azoospermic (n = 10). The tenets of the World Medical Association Declaration of Helsinki standards were adhered to in this study. Ethical Clearance was given by the University of Calabar Teaching Hospital Health and Research Ethical committee (UCTH/HREC/33/342). Informed written consent was given by the participants before being enrolled into the study. A standard questionnaire was used to obtain relevant information.

Inclusion and Exclusion criteria

Inclusion criteria

Men who have been married and staying together for over a year with no child/children, aged between 25-50 years who gave their consent were recruited as test subjects. Men who have fathered at least a child who gave their consent were included as controls

Exclusion criteria

Terminally ill persons, those with systemic diseases, who had undergone testicular surgery, chronic alcoholics, or heavy smokers, were excluded from the study. Men who have not had any child after at least one year of marriage with regular sex were excluded as controls

Sample collection

The participants were instructed to abstain from sex for 3-5 days. Semen samples were gotten by "self-help or masturbation" into a sterile Universal container (20ml) and brought to the laboratory within 30 minutes for analysis. After semen analysis, the semen samples were then centrifuged for 15 minutes at 4000rpm and seminal plasma obtained and was stored at -20^{0}C until analyzed.

Semen Analysis

On obtaining the semen, the volume of semen was recorded and viscosity checked, the samples

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were were allowed to liquefy at 37°C and semen was analyzed using a microscope and an improved Neubauer counting chamber according to standard guidelines (17). At least 200 cells were examined, and the sperm count, morphology, and percentage motility were determined. Normal sperm density was defined as greater than 20 million sperm/mL. The WHO's lower reference limit (5th percentile) is 15 million sperm per mL or 39 million sperm per ejaculate. But this is usually used as a reference for counseling couples and not used to label men as 'fertile' or 'infertile' (18). The semen samples were classified as normospermia indicated by sperm count > 20 million/ml, motility > 50%, and normal morphology > 40%, oligospermia indicated by sperm count < 20 million/ml irrespective of motility or morphology and azoospermia indicated by the complete absence of spermatozoa.[19]

Seminal plasma analyses

Seminal plasma total antioxidant capacity

Seminal plasma TAC was estimated by total antioxidant capacity assay kit obtained from (Rel Assay Diagnostics, Turkey). It involves measuring the reduction of 2,2'-azino-bis (3-ethyl-benzothiazoline-6-sulphonic acid; ABTS radical. Trolox was used to calibrate the assay and the results were expressed as mmol trolox Equiv/L.

Seminal plasma Vitamin C

Seminal plasma Vitamin C was estimated by the 2,4-dinitrophenylhydrazine method of Roe and Kuether.[20]

Seminal Plasma Catalase activity

Seminal Plasma Catalase activity was determined by titrimetric method as described by Udoh et al,.[21] Catalase was expressed as µmol of H_2O_2 degraded by enzyme/ml/min.

Seminal chlamydial IgG antibody detection

Seminal chlamydial IgG antibody detection was performed using the DRG chlamydial IgG antibody Enzyme linked Immunosorbent Assay Kit from DRG (GmbH, Germany). It is a solid-phase sandwich ELISA kit.

Seminal plasma Anti-Sperm Antibody ELISA test

Seminal anti-sperm antibodies quantitation was performed using the DRG Sperm Antibody Enzyme linked Immunosorbent Assay Kit from DRG (GmbH, Germany).

2.6 Statistical analysis

Data was analyzed by the SPSS 18 (Statistical Package for Social Sciences Inc, California, USA). The results were expressed as Mean ± Standard deviation. Analysis of variance (ANOVA) was used to analyze the data. Post hoc test was done using least significant difference (LSD). Significance level was set at p-value less than 0.05 ($p < 0.05$) at 95% confidence interval.

3.0 Results

Table 1 shows a comparison of age, semen parameters, seminal plasma total antioxidant capacity, catalase, vitamin C, antisperm antibodies and chlamydia in normospermic, oligospermic and azoospermic men. Sperm count, concentration, % sperm cell motility, total antioxidant capacity, catalase and antisperm antibodies varied significantly ($p = 0.000$) among the groups. The age of participants, semen volume and Vitamin C did not vary significantly ($p > 0.05$) among the groups. None of the participants were positive for antisperm antibodies and 4.4% of the infertile men were positive for chlamydia IgG antibody and all of them were from the oligospermic group i.e. 5.7% of the oligospermic men. The post hoc analysis showed that the oligospermic and azoospermic men had significantly lower ($p = 0.000$) sperm count, sperm concentration, % sperm motility,

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total antioxidant capacity, catalase and significantly higher (p = 0.002) anti sperm antibodies compared to the controls. Only the oligospermic men had significantly lower (p = 0.017) Vitamin C levels than the controls. The oligospermic men also had significantly higher % sperm cell motility (p = 0.0001) and catalase (p = 0.003) than the azoospermic men (Table 2). Catalase correlated positively and significantly with motility (r = 0.361, p = 0.017) and sperm count (r = 0.371, p = 0.014), while Vitamin C correlated negatively and significantly with Antisperm antibodies (r = -0.388, p = 0.009), Table 3.

Table 1:
Comparison of age, semen parameters, seminal plasma total antioxidant capacity, catalase, vitamin C, antisperm antibodies and chlamydia in normospermic, oligospermic and azoospermic men.

Parameters	Normospermic Fertile men (n = 35)	Infertile men		p - value
		Oligospermic n = 35	Azoospermic n = 10	
Age (yrs)	37.9 ±5.16	35.3 ± 5.73	38.1 ±6.21	0.110
Semen volume (ml)	2.5 ±0.91	2.7 ±1.32	1.9 ± 1.00	0.120
Sperm count (10^6/mL)	86.3 ±38.58	8.1 ± 5.52	0.00 ±0.00	0.000*
Sperm concentration (10^6)	217.1 ± 126.61	21.5 ±21.30	0.00 ±0.00	0.000*
% Motility	71.6 ±6.94	31.3 ± 11.59	0.00 ±0.00	0.000*
TAC (mmol/L Trolox equivalent)	0.59 ± 0.18	0.28 ± 0.18	0.33 ± 0.30	0.000*
Catalase (µmol/ml/min)	16.4 ± 4.82	11.3 ± 3.46	6.9 ± 2.21	0.000*
Vitamin C (mg/dl)	1.85 ± 0.92	1.25 ± 1.05	1.59 ± 1.06	0.057
Antisperm antibodies (U/ml)	3.46 ± 1.54	5.33 ± 1.70	5.39 ± 1.72	0.000*
Antisperm antibodies (% positive)	0(0)	0(0)	0(0)	-
Chlamydia (% positive)	0(0)	2(5.7)	0(0)	-

Results expressed as Mean ± SD, *significant at p < 0.05, TAC - Total antioxidant capacity

TABLE 2: Comparison of semen parameters, seminal plasma total antioxidant capacity, catalase, vitamin C, antisperm antibodies and chlamydia in normospermic, oligospermic and azoospermic men using post hoc analysis

Parameter	Groups		Mean Difference	p-value
	Normospermic men n = 35	Oligospermic men n = 35		
Sperm count (10^6/mL)	86.3 ±38.58	8.1 ± 5.52	78.203	0.000*
Sperm concentration (10^6)	217.1 ± 126.61	21.5 ±21.30	195.691	0.000*
% Motility	71.6 ±6.94	31.3 ± 11.59	40.286	0.000*
TAC(mmol/L Trolox equivalent)	0.59 ± 0.18	0.28 ± 0.18	0.314	0.000*
Catalase (µmol/ml/min)	16.4 ± 4.82	11.3 ± 3.46	5.051	0.000*
Vitamin C (mg/dl)	1.85 ± 0.92	1.25 ± 1.05	0.601	0.017*
Antisperm antibodies (U/ml)	3.46 ± 1.54	5.33 ± 1.70	-1.863	0.000*
	Normospermic men n = 35	Azoospermic men n = 10		
Sperm count (10^6/mL)	86.3 ±38.58	0.00 ±0.00	86.300	0.000*
Sperm concentration (10^6)	217.1 ± 126.61	0.00 ±0.00	217.164	0.000*
% Motility	71.6 ±6.94	0.00 ±0.00	71.571	0.000*
TAC(mmol/L Trolox equivalent)	0.59 ± 0.18	0.33 ± 0.30	0.257	0.000*
Catalase (µmol/ml/min)	16.4 ± 4.82	6.9 ± 2.21	9.470	0.000*
Vitamin C (mg/dl)	1.85 ± 0.92	1.59 ± 1.06	0.262	0.471
Antisperm antibodies (U/ml)	3.46 ± 1.54	5.39 ± 1.72	-1.927	0.002*
	Oligospermic men n = 35	Azoospermic men n = 10		

*significant at p < 0.05, TAC -Total antioxidant capacity

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Table 3:
Correlation of catalase and Vitamin C with some semen parameters in infertile men

Parameter	Index	r-value	p-value
Catalase	Sperm count	0.371	0.014*
	% sperm cell motility	0.361	0.017*
Vitamin C	Antisperm antibodies	-0.388	0.009*

*Significant at $p < 0.05$

4.0 Discussion

In this study, both the oligospermic and azoospermic men had lower sperm count, sperm concentration, % sperm motility, total antioxidant capacity, catalase. However, only the oligospermic men had significantly lower Vitamin C levels than the controls. This may be due to high levels of seminal plasma oxidative stress observed in the infertile men which depletes the antioxidants (22). Oxidative stress may be due to profuse production of ROS by the defective sperm or increased leucocytic activity triggered by genito-urinary infections (particularly of bacterial origin) and the consequent pro-inflammatory cytokine generation (23). Since central part of the sperm has the greatest concentration of antioxidants, a large portion of the sperm membrane is not protected leading to sperm dysfunction during massive OS owing to the peroxidative damage of lipid membranes and proteins as well as the DNA 23). This also agrees with studies by Huang et al. (24).

In this study, only 4.4% were positive for Chlamydia trachomatis IgG among the infertile males with all of them from the oligospermic group. This shows that though chlamydial infection may be a problem in our infertile males, it is not a major problem as the frequency is lower than that reported by Nwankwo and Sadiq (13) in Northwestern Nigeria (10.7%) and Enwuru and Umeh (14) in South eastern Nigeria (4.7%). There are contrasting views on the effect of chlamydial infection on seminal parameters; some studies have reported the absence of a marked relationship between major parameters of sperm analysis and the presence of seminal chlamydial IgG or IgA antibodies (25) while another study reported that seminal parameters were severely affected in infertile patients positive for seminal plasma anti-chlamydial lgG and lgA (26). We however could not assess its effect on seminal quality because of the small n umber (n =2) that were positive. However, demonstration of Chlamydial IgG antibodies in the male partner of an infertile couple is associated with a decreased probability of becoming pregnant (26). This is probably because there is a higher likelihood of the female partner being infected and suffering from the complications of chlamydial infections.

Antisperm antibodies (ASA) are a cause of male infertility and are produced when the blood-testis barrier is breached. However, it is not known if they are a major cause of male infertility in Nigeria. None of the infertile men were positive for ASA in this study, this suggests that ASA may not be the main factor of male infertility in our region. However, though the antisperm antibody levels of all the participants in this study were in the normal range the antibody levels of the infertile males were still higher than those of the fertile group. This has also been observed by other researchers who reported a 9-12% increase in ASA levels among infertile men (27). Though the reason for the presence of ASAs or their function in fertile men is not known, their presence in fertile men suggests that their mere presence may not disrupt fertility (28). We suggest that ASAs probably exert their effects at higher concentrations, or in persons who are

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susceptible due to the presence of favorable conditions such as oxidative stress, inflammatory conditions or even low levels of Vitamin C. Risk factors linked with the formation of ASAs in human males include breakdown of the blood–testis barrier, microbial infections, trauma from surgery, varicocele, orchitis, anal or oral sex, testicular and molecular mimicry toward some bacteria e.g. Escherichia coli (29). Our study showed a negative correlation between ASA and Vit. C levels. Supplementation with Vitamin C has been shown to have a protective role against anti-sperm antibody production in animal models (30). This may be because Vitamin C plays a very important role in the formation and maintenance of the basement membrane lining the capillaries and the intracellular cement that holds the endothelial cells together, thereby maintaining normal function and morphology of the testis. So Vitamin C directly protects against ASA production by its cellular protective effects ensuring the integrity of testicular cellular structure as well as hemotesticular barrier stability and indirectly through its powerful antioxidant action. Conversely, when the levels are low there is a higher probability of breaching the hemotesticular barrier triggering the production of ASAs. A study has also shown that supplementation with Vitamin C and E in infertile diabetic rats improved seminal characteristics.

This study has shown that the prevalence of chlamydia is 4.4% and that of seminal antisperm antibody 0% in the infertile males, levels of seminal antisperm antibody were increased while total antioxidant capacity, catalase, and vitamin C are decreased in infertile men compared to fertile men. Also, the levels of antisperm antibodies increased with a decrease in Vitamin C levels. Since depleted antioxidant status is associated with poor seminal quality this should therefore be addressed in the management of male infertility to increase better outcomes.

5.0 Conclusion

The prevalence of chlamydia and ASA in infertile men in this study was 4.4% and 0% respectively, seminal TAC, catalase, and Vitamin C were lower and ASA higher in infertile men than fertile men.

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