Original Article

Antichlamydia Antibodies and Sperm Quality among Male Partners of Infertile Couples in Nigeria

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Abstract

Background: The impact of Chlamydia trachomatis on semen quality has been studied with varied results. **Aim:** To determine the prevalence of antichlamydial antibodies and their relationship with sperm quality among male partners of infertile couples in Enugu, South-East Nigeria. **Materials and Methods:** It was a cross-sectional study of infertile male partners of couples attending infertility clinics at the University of Nigeria Teaching Hospital (UNTH) Ituku-Ozalla, Enugu, Nigeria. Their sera were assayed for antichlamydial antibodies, and semen analysis and culture were done for each participant. **Results:** Two hundred and eighty-two (282) male partners of infertile couples were studied. Infertility was commoner among participants aged 40 years or more (45.1%) and was mainly of the "primary type" (62.1%). Antichlamydia antibody was detected in 156 (55.3%) participants and was significantly associated with sperm quality (P = 002; OR = 2.294; 95% CI = 1.36–3.88). Overall, 81 (28.7%) had abnormal sperm quality. The sperm count, progressive motility, and vitality were significantly lower in participants with abnormal sperm quality than those with normal sperm quality ($P < 0.001$) while morphology, volume, and liquefaction time did not differ significantly ($P > 0.05$). *Staphylococcus aureus* was the predominant organism isolated from culture (122/282, 43.3%) while Streptococcus species were the least (4/262, 1.4%). There was significantly more *Staphylococcus aureus* isolated from the semen of participants that were seropositive to antichlamydial antibodies than those that were seronegative (80/156, 51.3% vs. 42/126, 33.3%; OR = 2.105; 95% CI = 1.30–3.42; P = 0.003). **Conclusion:** The prevalence of antichlamydial antibodies among male partners of infertile couples in Enugu, Nigeria is high and there is a significant association with sperm quality, sperm count, and bacterial isolates in seminal culture. Male partners of infertile couples in Enugu should be screened for antichlamydial antibodies and appropriate treatment offered wherever indicated. There is a need for increased public awareness and advocacy campaigns on the impact of Chlamydia infection on male factor infertility. This primary preventive measure may help in reducing the burden of Chlamydia infection and male factor infertility.

Keywords: *Antichlamydia antibodies, Chlamydia infection, male infertility, sperm quality*

Introduction

The burden of infertility is very high in sub-Saharan Africa and contributes to up to 65% of gynecological consultations in some localities.[1]

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Infertility due to male factors contributes to up to 50% of the cases.[2] Available evidence shows that semen quality is affected by many factors, including urogenital infections.[3] The impact of genital infections especially *Chlamydia trachomatis* on semen quality has been studied with varied results.[4-7]

Chlamydia infections often result in the development of an immune response and sometimes may lead to chronic infections and poor sperm quality.[8] African men run a greater risk of exposure to these infections partly due to poverty and partly due to inadequate health facilities to help in diagnosis and treatment.

The prevalence of *Chlamydia trachomatis* is influenced by racial, ethnic, and geographic variability.[9,10] Previous studies in Nigeria showed that one-third of male partners of infertile couples have abnormal semen characteristics, but little is known about the contributing factors to the observed abnormal semen qualities.[11-13]

In view of the asymptomatic nature of *Chlamydia infections*, the diagnosis of male infertility is rarely made by mere history or physical examination of suspected individuals.[4] It has been variously reported that infections caused by *Chlamydia* result in the formation of detectable antibodies in the serum of infected patients.[7,14,15] Therefore, screening individuals with male factor infertility for Chlamydia infection using antichlamydial antibodies could help in unraveling the possible etiological factor. This approach is very useful especially in low- and middle-income countries (LMICs) with a scarcity of facilities for Chlamydia tissue culture.[1]

This study was therefore aimed at determining the prevalence of antichlamydial IgG antibodies and the relationship with sperm quality among male partners of infertile couples in Enugu, South East Nigeria. The specific objectives were to determine the prevalence of antichlamydial antibodies among male partners of infertile couples, compare the seroprevalence of antichlamydial antibodies among male partners of infertile couples with abnormal semen quality with those with normal quality, and determine the association of antichlamydial antibodies with abnormal sperm quality among male partners of infertile couples.

Materials and Methods

This was a cross-sectional study of male partners of infertile couples presenting with infertility at the gynecology/infertility clinic of the University of Nigeria Teaching Hospital (UNTH) Ituku-Ozalla, Enugu, Nigeria, from January to December 2019. The hospital is the foremost federal tertiary health institution in Enugu, and it is situated at its permanent site at Ituku-Ozalla along the Enugu-Port Harcourt expressway at kilometer 21. The average number of gynecological patients, seen annually ranges between 5, 000 and 7, 000, and infertility accounts for 30% of the cases seen. The ratio of new to repeat cases in the clinics is approximately 1:3.

All the consenting eligible men who met the criteria were consecutively recruited from the population of male partners of infertile couples attending the clinic following individual counseling and informed written consent. The exclusion criteria were chronic medical diseases like diabetes mellitus, sickle cell disease, and hypertension, history of substance abuse or steroid medication, prior or current exposure to irradiation, history of urological surgery, history of past pubertal mumps, and history of smoking. For the women whose husbands were not present physically, but met the criteria for recruitment, the purpose of the study and its importance were discussed with them. The need to observe abstinence from any activity leading to ejaculation was highlighted and their husbands were given a short appointment in 3 days, and samples were subsequently collected from them. The recruited men were each given a pre-weighed sterile container for semen collection if they had abstained for at least 3 days before the presentation or after observing 3 days of abstinence. The semen was collected by masturbation after thoroughly washing their hands and genital area with water and drying with a clean towel.

The IgG antibody detection is a marker for a Chlamydia-positive immune response, either for current, chronic, or past infections. Thus, a positive result often indicates a past or chronic infection.[16] On the other hand, the detection of IgA antibodies is indicative of active infection.[16] Ideally, both IgG and IgA antibodies should be tested for a more accurate interpretation of results. However, in this study, only the IgG antibodies were tested for.

In other to test for the presence of IgG antibodies among the participants, 4 ml of venous blood was collected from their antecubital areas after cleaning with methylated spirit. The samples were centrifuged at 3000 r.p.m. The resulting sera were stored at -20°C in a deep freezer and were later analyzed in batches. Some participants were given transport fare where it was discovered it posed some challenges, to enable such husbands present at the clinic. The sera from the participants were assayed for antichlamydial IgG antibodies. This was done using "Xema Chlamydin IgG Elisa (96 W) – Lab Sakha (Chlamydia IgG EIA)"[16] which is a solid-phase enzyme immunoassay

for the qualitative determination of IgG antibodies to Chlamydia trachomatis in blood serum or plasma. It is marketed by Immunoconcept India Pvt. Ltd.[16] The proportion of participants seropositive for antichlamydial IgG antibody was determined.

Abnormal sperm quality was determined according to World Health Organization (WHO) 2010 criteria (minimum reference value) in terms of vitality (58%), morphology (4%), count (39 × 10^6 per ejaculate), and motility (40%) and was defined as the finding of abnormality in one or more of the parameters.[17]

Table 1: Socio-demographic Characteristics of the study participants

	Frequency	Percent
Age group		
20–24	11	3.9
25–29	38	13.5
30–34	57	20.2
35–39	49	17.4
40–44	86	30.5
45–49	14	5.0
50 & above	27	9.6
Marital status		
Married	256	90.8
Divorced	3	1.1
Separated	23	8.2
Ethnic group		
Igbo	245	86.9
Hausa	15	5.3
Yoruba	8	2.8
Others	14	5.0
Religion		
Christian	258	91.5
Moslem	21	7.4
Traditional	3	1.1
Employment		
Employed	235	83.3
Not employed	47	16.7
Occupation		
Civil service	158	56.0
Artisan	89	31.6
Others	35	12.4
Educational level		
No formal	6	2.1
Primary	39	13.8
Secondary	136	48.2
Tertiary	101	35.8
Period of infertility		
1yr	90	31.9
>1yr	192	68.1
Previous pregnancy		
None	175	62.1
1 or more	107	37.9

Table 2: Pattern of sperm characteristics of the study participants

	Frequency	Percent
Liquefaction time		
Normal	282	100.0
Abnormal	-	-
Viscosity		
Normal	236	83.7
Abnormal	46	16.3
Volume		
Normal	239	84.8
Abnormal	43	15.2
Progressive motility		
Normal	177	62.8
Abnormal	105	37.2
Vitality		
Normal	147	52.1
Abnormal	135	47.9
Sperm count		
Normal	178	63.1
Abnormal	104	36.9
Morphology		
Normal	264	93.6
Abnormal	18	6.4

The sample size was determined using the formula, $n = Z^2pq/d^2$, where *P* was set as 24% from a previous study carried out in Benin City, Nigeria,[18] and a 10% attrition rate. Thus, the calculated minimum sample was 308.

The ethical clearance approval was obtained from the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu Research Ethics Committee (UNTH/CSA.329/Vol 5). A written informed consent was obtained from each participant before recruitment into the study. Data was collected using a structured proforma designed for the study. This sought information regarding the socio-demographic characteristics of the subjects, period, and type of infertility as well as the history of a prior pregnancy. It was also used to collect data on the outcome of the laboratory investigation results. The primary outcome measures were the prevalence of antichlamydial antibodies among male partners of infertile couples and the proportion of participants with abnormal sperm quality who are seropositive to *Chlamydia* antibodies. The secondary outcome measures were the proportions of male partners of infertile couples who had abnormal sperm parameters. Data were analyzed with a statistical package for the social sciences (SPSS) software, version 24.0, IBM SPSS, Chicago, Illinois. A comparison of means of continuous variables was done using Student's *t*-test. Associations between categorical variables were done using Chi-square or

Fisher's exact. Relationships were expressed using odd ratios and confidence intervals. A *P* value less than 0.05 was considered to be statistically significant.

Results

A total of 308 eligible men were recruited for the study, however, 26 men (8%) had equivocal antichlamydial antibody results and were discarded for analysis. Thus, 282 (91.6%) participants were used for the study. The majority of the participants were aged between 40 and 44 years (30.5%). Two hundred and fifty-six (90.8%) were married, while 3 men (1.1%) and 23 (8.2%) were either divorced or separated, respectively. Most participants were of the Igbo tribe (245; 86.9%) and predominantly Christians (258; 95%). Also, the majority were employed (235; 83.3%) and civil servants (158; 56.0%). The highest level of education was secondary representing 48.2% (136) of the study population. Predominantly, 62.1% (175) had primary infertility. Details of the socio-demographic variables of the participants were as shown in Table 1.

The overall prevalence of IgG antichlamydial antibody was 55.3% (156/282, 55.3%). A total of 81 participants (81/282, 28.7%) had abnormal sperm quality, while 201 (201/282, 71.3%) had normal sperm quality. The proportion of participants with abnormal sperm quality who were seropositive to antichlamydial was 60.5% (49/81, 60.5%) while that with normal sperm quality was 39.3% (79/201, 39.3%). The observed difference was statistically significant (OR = 1.80; 95% = 1.1–3.0; *P* = 0.02).

Table 2 shows the pattern of sperm parameters among the study population. Overall, 63.1% had normal sperm count, while 62.8% and 93.6% had normal progressive motility and normal morphology, respectively. Details are shown in the table.

Table 3 shows that progressive motility, vitality, and sperm count were significantly lower in participants with abnormal sperm quality than in those with normal sperm quality ($P < 0.001$). However, there was no significant difference in terms of liquefaction time, volume, and morphology between participants with abnormal and normal sperm quality ($P > 0.05$).

Table 4 shows that antichlamydial antibodies were significantly associated with abnormal sperm count ($P = 0.02$). Other parameters including liquefaction time, volume, progressive motility, vitality, and morphology did not show any significant association with antichlamydial antibodies ($P > 0.05$).

Table 5 shows that *Staphylococcus aureus* was the predominant organism isolated from the culture in both participants with abnormal and normal sperm quality, while Streptococcus species were the least. Also, significantly more *Staphylococcus aureus* was isolated from the semen of participants seropositive to antichlamydial antibodies than those that were seronegative ($P = 0.003$).

Other isolated bacteria growths did not have any significant association with antichlamydial antibody status ($P < 0.05$). Details are shown in Table 5.

Discussion

The study demonstrated that the prevalence of antichlamydial IgG antibodies among male partners of infertile couples was 55.3%, while the prevalence of abnormal sperm quality was 28.7%. It also revealed that 60.5% of male partners of infertile couples with abnormal sperm quality were seropositive to antichlamydial IgG antibodies. These observations corroborated earlier reports that antichlamydial antibodies are associated with male infertility and implicated in abnormal sperm characteristics.[4,7,14,15] The 55.3% prevalence of antichlamydial IgG antibody obtained in this study is higher than the 24% obtained from Benin City,[18] South-South Nigeria, 37.1% from Morocco,[15] North Africa, and 21.5% from the USA,[19] corroborating the suggestion that Chlamydia infection shows ethnic, geographic, and racial variability.[9,10,20,21] It may also be due to the different types of antichlamydial antibody kits used with different sensitivities and specificities that

Table 3: Comparison of mean semen parameters between participants with abnormal and normal sperm quality

Sperm parameters	Sperm quality		*t*-test	*P*
	Abnormal Mean±SD	Normal Mean±SD		
Liquefaction Time	33.79±4.45	34.88±4.68	1.836	0.067
Volume	2.85±1.63	3.23±1.16	1.933	0.054
Progressive motility (%)	30.43±22.54	58.68±10.01	10.845	<0.001
Vitality	42.08±27.27	75.31±11.47	10.584	<0.001
Sperm count	26.58±23.65	48.43±19.74	7.332	<0.001
Morphology normal form	73.51±25.26	78.89±11.24	1.844	0.066
Morphology abnormal form	17.09±11.91	19.44±9.22	1.594	0.112

Table 4: Association between anti-chlamydial antibodies and sperm parameters among participants with abnormal sperm quality

	Antichlamydial antibodies		χ^2	*P*
	Present No. (%)	Absent No. (%)		
Liquefaction Time				
Normal	123 (61.2)	78 (38.8)	NA	NA
Abnormal	0 (0.0)	0 (0.0)		
Viscosity				
Normal	99 (63.9)	56 (36.1)	2.044	0.153
Abnormal	24 (52.2)	22 (47.8)		
Volume				
Normal	96 (60.8)	62 (39.2)	0.059	0.809
Abnormal	27 (62.8)	16 (37.2)		
Progressive motility				
Normal	54 (56.3)	42 (43.8)	1.892	0.169
Abnormal	69 (65.7)	36 (34.3)		
Vitality				
Normal	36 (54.5)	30 (45.5)	1.829	0.176
Abnormal	87 (64.4)	48 (35.6)		
Sperm count				
Normal	51 (52.6)	46 (47.4)	5.862	0.015
Abnormal	72 (69.2)	32 (30.8)		
Morphology normal form				
Normal	111 (60.7)	72 (39.2)	0.249	0.618
Abnormal	12 (66.7)	6 (33.3)		

χ^2=Chi square; NA=Not Applicable

Table 5: Microorganisms isolated from semen of male partners of infertile couples

	Sero-positive *n*=156 No. (%)	Sero-negative *n*=126 No. (%)	χ^2	*P*
Staph sp	80 (51.3)	42 (33.3)	16.392	0.003
Strep sp	0 (0.0)	4 (4.2)	-	-
Klebsiela sp	6 (3.8)	3 (2.4)	0.126	0.722
E. coli	19 (12.2)	15 (11.9)	0.005	0.944
Coliform sp	28 (17.9)	20 (15.9)	0.091	0.763

χ^2=Chi square

detected antichlamydial antibodies either qualitatively or quantitatively.[16] The 28.7% prevalence of abnormal sperm quality is similar to the findings of 27.3% from Ibadan, South-West Nigeria.[12] However, this was less than 64% from Abakaliki, South-East Nigeria,[13] and 58.5% from Morocco,[15] North Africa. The similarity of the sperm quality finding with that of the Ibadan study may be due to the cosmopolitan nature of both cities.

Overall, the proportion of men with abnormal sperm quality who were seropositive to antichlamydial antibody was 60.5% compared to 39.3% of those with normal sperm quality who were seropositive to antichlamydial antibody. In this study, the antichlamydial antibody was significantly associated with sperm quality. These findings were similar to some previous related studies[6,7,22] but also in contrast with some previous related studies.[23,24] These disparities may be due to regional and racial differences and varied detection techniques and characteristics of the study population.[10]

Staphylococcus aureus was the commonest organism isolated from the study. This was similar to the findings from a previous study in Ile-Ife, South-West Nigeria.[11] However, the specific impact of these organisms could not be confidently demonstrated in this study. Hence, further study designs will be necessary in the future to evaluate this. There was a significant association of bacterial culture of *Staphylococcus aureus* with positive antichlamydial antibody. This indicates that participants with positive antichlamydial antibodies were at increased risk of being infected with *Staphylococcus aureus*. However, other microorganisms did not show a significant association. The reason for the observed increased susceptibility to *Staphylococcus aureus* infection compared to other microorganisms could not be demonstrated in this study, and future studies may consider this direction.

The limitations of this study included the fact that the analysis of IgG antibody was done in batches using thawed samples (96 microwells/strips per batch) and thus loss of quality with long storage of specimens could not be completely ruled out.

Also, the study could not demonstrate the Chlamydia trachomatis serotypes associated with abnormal sperm quality. Thus, further follow-up studies would be necessary in the nearest future to unravel this. Despite the high rate of bacterial isolates observed in this study, the inability to determine in detail their association with abnormal sperm quality was a limitation. Also, this study accessed for any "casual" associations between antichlamydial IgG antibodies and sperm characteristics and positive bacterial isolates. However, a more robust or closed association would also require an assessment of the IgM antibodies and Chlamydia isolates in tissue culture. Future studies should therefore consider this direction.

Conclusion

The prevalence of antichlamydial antibodies among male partners of infertile couples in Enugu, Nigeria is high and there is a significant association with sperm quality, sperm count, and bacterial isolates in seminal culture. It is therefore recommended that male couples of infertile couples in Enugu be screened for antichlamydial antibodies and appropriate treatment

offered wherever indicated. There is also a need for increased public awareness and advocacy campaigns on the impact of Chlamydia infection on male factor infertility. This primary preventive measure may help in reducing the burden of Chlamydia infection and male factor infertility.

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Conflicts of interest

There are no conflicts of interest.

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