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The Effect ofProlactin, Total Antioxidant Capacity, Antisperm Antibodies andthePresence of *Chlamydia trachomatis* IgGinFertile andInfertile Women inCalabar, Nigeria

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Abstract

Aim: *Chlamydia is a common sexually transmitted disease (STD) caused by the bacterium, Chlamydia trachomatis (CT), which can damage a woman's reproductive tissues, thus, affecting fertility. Our aim therefore was to determine the total antioxidant capacity (TAC) andantisperm antibodies (ASA) in relation to the presence or absence of Chlamydia trachomatisIg G in fertile and infertile women.*

Materials and Methods:*One hundred and two (102) volunteer infertile women visiting the University of Calabar Teaching Hospital and sixty (60) apparently healthy women (control group), aged 20 – 45years were recruited. The infertile women were further grouped as primary (n = 14) and secondary (n = 88) infertility as appropriate and also divided into three groups based on their prolactin levels, namely; those with normal value (normal) (n=32), those with prolactin values between 25-60ng/ml (moderate) (n=46) and those with prolactin values >60ng/ml (high)(n=24). Serum samples were obtained from the subjects and Chlamydia trachomatisIg G and ASA assays were done using ELISA method while Serum total antioxidant capacity measurement was done spectrophotometrically.*

Results: *The mean prolactin and total antioxidant capacity of the infertile group was significantly higher than that of the fertile group ($p<0.05$) while there was a negative correlation between TAC and prolactin in infertile women ($r=-0.196$; $p<0.05$).No significant difference ($p>0.05$) was found in the total antioxidant capacity and antisperm antibodies between the infertile women with normal hormonal profile and the controls, while prolactin was high significantly ($p<0.05$) in the infertile women with normal ovarian profile than the controls. The prolactin values were significantly higher in the high group compared with the normal ovarian profile and moderate group. Also, the antisperm antibodies levels were significantly higher in the moderate group compared to the high and normal groups ($p<0.05$).*

Conclusion:*Chlamydia trachomatis infection is low while total antioxidant capacity is higher among the infertile women in our test population. This study shows that antioxidant is involve in infertility and may have potential application in diagnosis and treatment of female infertility.*

Keywords:*Total antioxidant capacity (TAC), antisperm antibodies (ASA), Chlamydi trachomatisIg G, fertile, infertile women.*

I. Introduction

Infertility is defined as the inability of a couple to achieve conception despite frequent unprotected, well timed sexual intercourse for one year duration. It also includes the inability of a woman to carry a pregnancy to the delivery of a live baby (1). Infertility occurs in one out of five couple of reproductive age and in ten to twenty per cent of these cases, there seems to be no definitive cause, therefore these are classified as unexplained infertility (2, 3).

Globally, the highest incidence of infertility is found in Central and Southern Africa where as many as 1 in 3 women are infertile (4, 5). The primary cause of infertility worldwide is tubal disease due to infection, including Gonorrhoea, Chlamydia infection, and tuberculosis (4). A retrospective study done by Ekwere *et al.* (6), on patients coming to the Obstetrics and Gyneacology clinic in Calabar, the causes of infertility was 58% female, 30% male while 12% were both causes. Primary infertility was found in 69.7% of the males and 34.5% of the female .while secondary infertility was30.3% in males and 65.5% in females; Infection being the strongest predisposing factor. In our locality routine infertility checkup is limited to ovarian causes alone i.e. in the use of 3rd day follicle stimulating hormones and 21st day progesterone and other fertility profile hormones. The tubal axis of infertility is usually done using hysterosalpingiography which is costly, invasive with its attendant problems of infection, abortion, peritoneal reaction and generalized sensitivity. Therefore there is need

to identify and ascertain non - invasive but equally sensitive techniques of determining the total antioxidant capacity (TAC), antisperm antibodies (ASA) and the presence of *Chlamydia trachomatis*Ig G in fertile and infertile women.

II. Materials And Methods

A group of women attending the infertility clinic in the Department of Obstetrics and Gynecology and coming for infertility test in the Department of Chemical Pathology of the University of Calabar Teaching Hospital (UCTH) were selected for the study. The selection of patients was done with the help of a Gynaecologist in the Obstetrics and Gyneacology Department of the hospital. One hundred and two (102) volunteers infertile women, aged 20-45 years were recruited and further grouped as primary and secondary infertility as appropriate. Fourteen of the women had primary infertility while 88 of them had secondary infertility. Sixty (60) apparently healthy, age matched women who had given birth to at least one child within the last three years, were selected to serve as the control group. Approval was given from the Health Research Ethical Committee (HREC) of the Hospital and the subjects all gave informed consent to participate in the study. Their confidentiality was maintained. Inclusion criteria used was that apparently healthy women with infertility problems and healthy women who had given birth to at least one child within the last three years while women who were pregnant or older than 45 years of age and those with high blood pressure and diabetes were excluded.

Five milliliters (5ml) of venous blood was obtained from these test subjects in the luteal phase (21^{st} – 23^{rd} day) of their menstrual cycle into clean plain bottles (containing no anticoagulants). The blood was allowed to clot and was centrifuged at 3000rpm for five (5) minutes. The serum was then separated by the use of pasteur pipettes into serum containers with tight screw caps and was stored at -20^{o}C in aliquots of one milliliter until ready for use. The samples were used for Prolactin, *Chlamydiatrachomatis*Ig G and antisperm antibodies assay using ELISA method. Serum total antioxidant capacity measurement was done using the total antioxidant status assay kit [RL0017 (product code); Rel Assay Diagnostics, turkey].

III. Data Analysis

The data was analysed using Microsoft Excel and PASW (Predictive Analysis Software) version 18 packages from SPSS Inc. USA.

IV. Results

Their mean ages where 31.1±5.37 and 33.1±4.91years respectively. The infertile women were further divided into three groups based on their prolactin levels; namely those with normal ovarian hormones value (normal); (n=32), those with prolactin values between 25 – 60ng/ml (moderate) (n=46) and those with prolactin values greater than 60ng/ml (high); (n=24).

Table 1 shows the comparison of prolactin, antisperm antibodies and total antioxidant capacity levels in fertile and infertile women. There was no significant difference ($p>0.05$) in the antisperm antibodies levels between the fertile and infertile group. The mean prolactin and total antioxidant capacity of the infertile group was significantly higher than that of the fertile group ($p<0.05$).

Table 2 shows comparison of prolactin, antisperm antibodies and total antioxidant capacity level in infertile women with normal hormonal profile and fertile control. There was no significant difference ($p>0.05$) in the total antioxidant capacity and antisperm antibodies between the infertile women with normal hormonal profile and the controls. However, the prolactin was significantly higher ($p<0.05$) in the infertile women with normal ovarian profile than the controls.

Table 3 shows the comparison of prolactin, antisperm antibodies and Total Antioxidant Capacity (TAC) between infertile women groups of normal ovarian profile, moderate and high prolactin. Here, the prolactin values were significantly higher in the high group compared to the normal ovarian profile and moderate group. Also, the antisperm antibodies levels were significantly higher in the moderate group compared to the high and normal groups ($p<0.05$). There was no significant difference in the value of TAC among the groups ($p>0.05$).

Table 4 shows the percentage of *Chlamydia trachomatis* in fertile and infertile women. Out of the 102 infertile subjects, 8 (8.2%) were positive for Chlamydia IgG and 94 (91.8%) were negative while 6 (10%) of the fertile controls was positive for Chlamydia IgG and 54 (90%) were negative (n=60). It shows significant difference among the group when subjected to a statistical tool.

Figure 1 shows a negative correlation plot ($r=-0.320$; $p<0.05$) between antisperm antibodies and prolactin in the control group. Figure 2 shows a negative correlation graph of TAC against prolactin in the control group. ($r=-0.422$; $p<0.05$). In Figure 3, the correlation of TAC against prolactin in the test group was negative ($r=-0.196$; $p<0.05$).

TABLE 1 Comparison of prolactin, antisperm antibodies and total antioxidant capacity level in fertile and infertile women

Parameters	Fertile	Infertile	Calc"t"	Crit"t"	P-value
Prolactin(ng/ml)	9.16±12.05	45.6±36.98	7.38	1.97	p<0.05
Antisperm(U/ml)	88.8±27.31	91.1±34.81	0.45	1.97	p>0.05
TAC(mmolTROLOXEQIV/L)	1.09±0.22	1.26±0.33	3.39	1.97	p<0.05
N	60	102			

Values are mean±SD

TABLE 2 Comparison of prolactin, ASA and TAC level in infertile women with normal hormonal profile and fertile control

Parameter	Fertile (Control)	Infertile (Normal Profile)	Calc "t"	Crit "t"	P-value
Prolactin(ng/ml)	9.2±1.6	15.5± 0.86	2.86	1.99	p<0.05
Antisperm(U/ml)	88.8±3.5	79.5±4.36	1.6	1.99	p>0.05
TAC(mmolTROLOXEQIV/L)	1.09±0.28	1.20±0.65	1.77	1.99	p>0.05
N	60	32			

Values are mean±SD

TABLE 3 Comparison of prolactin, ASA and TAC among the infertile women groups

Parameter	Normal Prolactin	Moderate Prolactin	High Prolactin	Calc "f"	Crit "f"	P-value
Prolactin(ng/ml)	15.5±4.86	35.7±9.98	104.4±26.68	267.2	3.088	p<0.05
Antisperm(U/ml)	79.5±24.66	102.4± 42.89	84.9±20.91	4.937	3.088	p<0.05
TAC (mmolTROLOX EQIV/L)	1.20±0.37	1.34±0.35	1.16±0.17	2.144	3.088	p>0.05
N	32	46	24			

Values are mean±SD

TABLE 4 The percentage of *Chlamydia trachomatis* in fertile and infertile women

Group	Positive	Negative	N
Infertile	8 (8.2%)	94 (91.8%)	102
Fertile	6 (10%)	54 (90%)	60

p>0.05

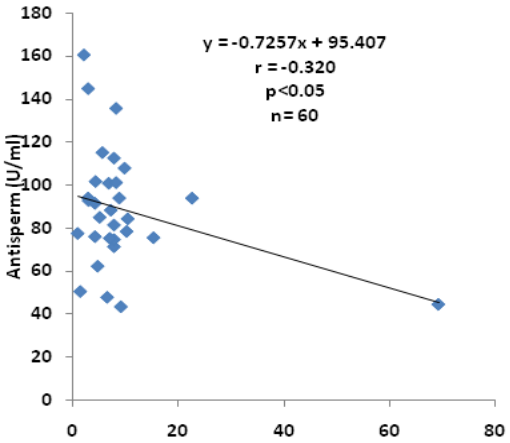

Figure 1: Correlation graph of antisperm antibodies against prolactin in the control group (fertile). There was a negative correlation. (r = -0.320; p<0.05).

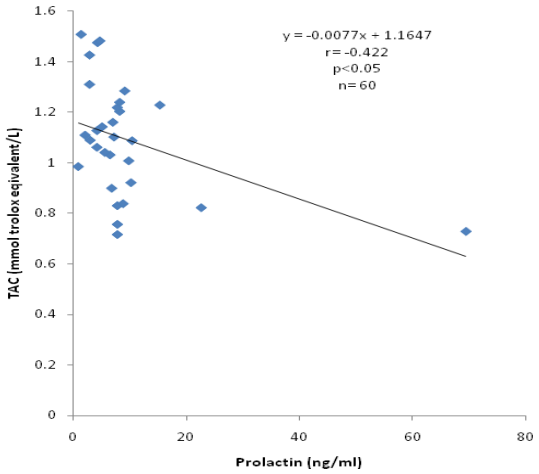

Figure 2: Correlation graph of TAC against prolactin in the control group (fertile). There was a negative correlation. (r =-0.422; p<0.05).

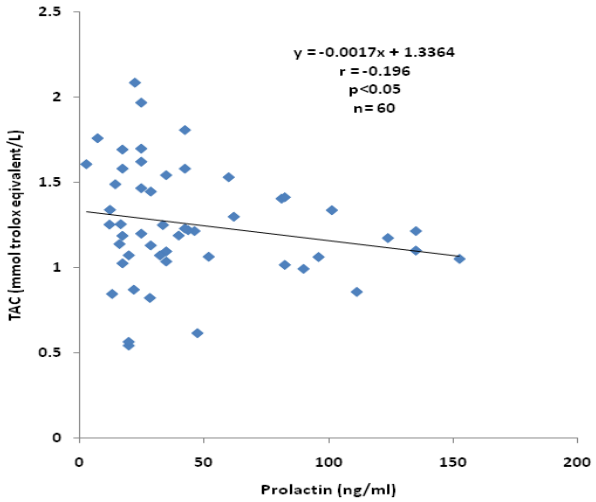

Figure 3: Correlation graph of TAC against prolactin in the test group (Infertile). There was a negative correlation. (r =-0.196; p<0.05).

V. Discussion

This study was conducted to determine and compare serum levels of prolactin, antisperm antibodies (ASA), total antioxidant capacity (TAC) and *Chlamydia trachomatis*Ig G in fertile and infertile women. In our study there was no significant difference in the levels of ASA between the fertile and infertile groups (p>0.05) this is contrary to work done by Mutar*et al.* (7) and Siam & Hefzy (8) who reported significantly higher mean antisperm antibody in serum of infertile patients when compared to fertile control, however interestingly, when compared among the infertile groups, the levels of ASA in the group with moderately raised prolactin levels was significantly higher than both the normal and high prolactin groups (p<0.05). This may be due to the fact that prolactin at moderate levels enhances antibody production and release but as the concentration of prolactin increases, prolactin may reduce the expression of Ig Greceptors. There was a negative correlation between ASA and prolactin in the controls. This is in contrast to previous studies who observed a positive relationship between them because in mice, Bernton *et al.* (9) found that prolactin enhances antibody production and release, thus prolactin helps to maintain lymphocyte function and lymphokine-dependent macrophage activation.

Work done by Gleicher (10), suggests that hyperprolactinaemia may affect fecundity not only by affecting ovulation and implantation through hormonal but also through immunological processes. This is because the high incidence of hyperprolactinaemia in women with infertility problems mimicked the presence of significant autoimmune abnormalities. But Kawaguchi *et al.* (11) from their findings suggested that antisperm antibody may have an inhibitory effect on prolactin secretion. This is in consonance with our findings. Work by Barrington *et al.* (12) had serum PRL levels negatively correlated to mammary secretion IgG1 concentrations

and IgG1 receptor score. The significant negative correlation between serum PRL and IgG1 receptor suggests that bovine mammary IgG1 receptor expression is reduced by exposure to PRL, *in vitro.*

Chlamydia is a common sexually transmitted disease (STD) caused by the bacterium, *Chlamydia trachomatis* (CT), which can damage a woman's reproductive organs. Studies from the Netherlands shows that having antibodies against Chlamydia is a potent predictor of blocked tubes and many women infected with Chlamydia don't have high antibody titers to *Chlamydia* (13). A WHO (14) study reported *Chlamydia* infection in infertile women to be 18 – 20 %. In India, *C. trachomatis* was detected in 31 (28.1%) of the 110 infertile women while one (3.3%) in control group was positive for *C. trachomatis* ($p<0.01$) (15).

In our present study, chlamydia infection was detected in 8 (8.2%) of the 102 infertile women, while six (10%) in the control group of 60 women was positive for C. trachomatis. The prevalence obtained in this study were lower than those obtained in other studies by Ikeme *et al.* (16) at Enugu, who reported a prevalence of 29.4% among female residents and Inyang - Etoh*et al*.(17) who reported a prevalence of 22% in infertile women in Calabar. This marked difference could be as a result of a more sensitive method used i.e quantitative analysis not qualitative as previously used, also reduced sexual risk-behaviour, increased awareness of Chlamydia infection and other sexually transmitted diseases, easy access to laboratory, diagnoses and treatment.

Fadwa (18) had a prevalence of CT to be 25% in infected women, while Siemer *et al.* (19) had a prevalence of 33% in infertile women in Ghana. Vidwan*et al.* (20) in one of the studies on CT prevalence amongst healthy pregnant mothers in southern India documents a very wide variation in CT prevalence. With prevalence as low as 3.3% in Velline, 0.5 % in Munbai, and New Delhi had a prevalence of 28%. Many false positive results were noted using the rapid test. In Iran, Badami and Salari (21) studied the frequency of CT in 125 infertile female. *Chlamydia trachomatis* was detected by direct IF in 11 (8.8%) of infertile and 2 (0.8%) control group. The rate of *Chlamydia trachomatis*, in case and control groups was significant ($p<0.0001$). Belongia *et al*. (22) noted a geographic variation in the rate of *Chlamydia* infection, with *C. trachomatis* infection increased in rural areas and this they explained may be as the result of differences in sexual habits and socioeconomic status between rural and urban areas.

In our present study the levels of total antioxidant capacity (TAC) contrary to most documented studies was higher in infertile subjects than in the controls ($p<0.05$). This was higher especially in subjects that had moderately raised prolactin. But when compared among themselves, the levels of TAC were not statistically different ($p>0.05$). These findings is contrary to Prieto *et al*. (23), Polak *et al*. (24) and Szczepanska *et al.* (25), who had total antioxidants lower in infertile women than in fertile controls. While Wang *et al.* (26) had no difference in the antioxidant levels in the peritoneal fluids of women with endometriosis and fertile controls. Our findings is in agreement with work done by Veena *et al.* (27), who observed higher concentration of ferric reducing antioxidant power (FRAP) in infertile women when compared to fertile controls. Also higher concentration of nitrite was found in controls than in infertile patients. Alpay *et al*. (28) reported increased expression of copper/zinc supra oxide dismutase in infertile women with endometriosis. This increase in total antioxidant observed in the infertile subjects may be due to several factors which include the use of food supplements and the routine administration of certain antioxidants like vitamin E and C for infertility treatment. Studies by Foyouzi *et al.* (29), showed that high levels of various antioxidants inhibit the proliferation of endometrial stromal cells and that moderate levels of oxidative stress promote endometrial stromal cell proliferation. It was also found that excessive amount of vitamin antioxidant can trigger oxidative stress damage (29).Heavy consumption of carotene-containing vegetables may cause amenorrhoea by increasing faecal excretion of oestrogens and thus decreasing blood levels of estradiol (30). Large doses of ascorbic acid may be associated with inhibition of ovarian steroidogenesis (31), reduced fertility (32) and increased probability of abortion (33).

A negative correlation between total antioxidant capacity and prolactin was seen in both infertile and fertile subjects. Veena *et al.* (27) observed a positive correlation between serum prolactin and nitrite (which is an oxidative stress marker) this results suggests that hyper prolactinemia could contribute to infertility by inducing oxidative damage. Antioxidant enzymes, including catalase, form the first line of defence against free radicals; therefore their regulation depends mainly upon the oxidant status of the cell. However, there are other factors involved in their regulation, including the enzyme-modulating action of various hormones such as growth hormone, prolactin and melatonin. Growth hormone, and possibly prolactin, was found to decrease catalase and other antioxidant enzymes in various tissues in mice, suggesting that this hormone acts as a suppressor of key antioxidant components (11).

VI. Conclusion

From our study we observed that *Chlamydia trachomatis* infection is low in infertile women in our test population while Total antioxidant capacity is higher among the infertile subjects. These findings suggest involvement of antioxidant in infertility and thus may have potential application in diagnosis and treatment of female infertility.

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