CHLAMYDIAL INFECTION, PLASMA PEROXIDATION AND OBESITY IN TUBAL INFERTILITY

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ABSTRACT

Background: Genital tract infections and obesity are both sources of oxidative stress. Alterations in immune and antioxidant parameters may arise from this or from an indeterminate autoimmune mechanism.

Objective: This study aimed to investigate the association of Chlamydial infection, obesity and oxidative response with tubal infertility in Nigerian women.

Methods: It was a case-control study of 40 women with tubal infertility and 32 fertile women, respectively, recruited from the Infertility and Family Planning Clinics respectively, of the University College Hospital, Ibadan, Nigeria. Anthropometric indices were measured in each subject and endocervical swabs were taken to screen for current genital tract infection. Antioxidant, hormonal and immunologic analysis were performed on serum.

Results: None of the subjects had current genital tract infections. Chlamydia trachomatis IgG positivity was significantly higher in infertile than in fertile subjects [OR 4.33; 95%CI (0.078-0.681)]. No significant variations were observed in the anthropometric indices, antioxidant parameters and hormones between infertile and the fertile women. Body mass index correlated positively with oxidative stress in infertile subjects. Waist and hip circumferences correlated negatively with oestradiol in women with tubal infertility.

Conclusion: Chlamydial infection is associated with tubal factor infertility, however, obesity seems to increase oxidative stress and reduce fertility potential in women with tubal factor infertility.

Key words: Tubal infertility, obesity, oxidative stress, Chlamydia

INTRODUCTION

Human reproductive failure is as old as mankind. This public health problem involves all regions of the world and its prevalence worldwide varies from 10-15%; 10-32% in Africa² and 31.5% in Nigeria³. The various categories of human reproductive failure include: infertility, recurrent pregnancy loss (spontaneous miscarriages or abortions and preterm birth) and ectopic pregnancy. Causes include environmental and life style factors, congenital malformations, endocrine disorders, immunologic abnormalities and sequelae of genital tract infections⁴⁵.

The association of infertility with genital tract infections (GTIs) or sexually transmitted infections has been demonstrated. GTIs associated with human reproductive failure include: Treponema pallidum, Neisseria gonorrhoea, Chlamydia trachomatis, Trichomonas vaginalis and Schistosoma haematobium infections among others. The spread of gonococcal and Chlamydial infection to upper female genital tract may cause pelvic inflammatory disease with severe tubal scarring leading to tubal infertility⁶. Genital tract infections evoke both cellular and humoral immune response leading to activation of polymorphonuclear leukocytes, macrophages and the release of cytokines. These immunologic factors produced in response to GTIs influence various aspects of reproduction including follicle development, ovulation, luteinisation, oocyte quality, fertilization, implantation, foetal development and pregnancy immunotolerance⁷⁻⁹.
This study aimed to investigate the association of Chlamydial infection, obesity and oxidative response with tubal infertility in Nigerian women.

**MATERIALS AND METHODS**

**Study design**

This case control study was conducted in the University College Hospital (UCH), Ibadan, Nigeria. It served as a pilot for an on-going larger work studying the association of oxidative stress and pathologic response to stress with infertility. The study population comprised of female patients of reproductive age attending the Infertility Clinic and a control group of age-matched fertile women who were new clients at the Family Planning Clinic. The study protocol was approved by UI/UCH Ethical Committee (Ref UI/EC/08/0083).

**Hypothesis**

The hypothesis was that tubal infertility is not associated with oxidative stress and obesity. The outcome measures were the anthropometric and oxidative stress parameters of the subjects.

**Selection of Subjects**

Forty consenting women with infertility of at least one year’s duration (with tubal blockage identified by hysterosalpingography) were recruited from the infertility clinic, while 32 controls were recruited from the family planning clinic. The controls were women without previous infertility who had childbirth within the last two years, and who had not been on any form of contraception prior to recruitment. Exclusion criteria included women that were undergoing any form of contraceptive therapy, previous history of uterine surgery, malignancy, long term medication, chronic organ or systemic illness and those who did not give consent.

Anthropometric indices (height, weight, waist and hip circumferences) were taken to calculate the body mass index and waist-hip ratio (WHR) respectively.

**Sample Collection**

Ten milliliters of venous blood samples were collected aseptically from each subject on days 3-5 and 21-23 of a 28-30 day menstrual cycle, respectively. The samples were dispensed into universal containers. After clot retraction, the samples were centrifuged at 3000 rev/s for ten minutes after which serum was extracted and stored in small aliquots at -20°C. High vaginal swabs (HVS) and endocervical swabs (ECS) were also taken from all subjects of study using sterile swab sticks for detection of common sexually-transmitted infections.
Laboratory Methods
Endocrinological analysis: follicular stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P4), estradiol (E2) and prolactin (PRL) were measured with enzyme immunoassay method (EIA) from Immunometrics Ltd., London, UK.

Antioxidant profile: total antioxidant potential (TAP) was estimated using the ferric reducing antioxidant power (FRAP) method of Benzie and Strain\textsuperscript{24}. Total plasma peroxides (TPP), as a biomarker of Oxidative Stress (OS), were estimated using the modified FOX 2 method of Harma \textit{et al.}\textsuperscript{25}. Oxidative stress index (OSI) was calculated as a ratio of TPP/TAP.

Microbiological Analysis: screening for \textit{Trichomonas vaginalis} was by microscopy while \textit{Neisseria gonorrhoeae} was by gram staining, followed by culture if necessary. Immunologic analysis: endocervical swabs were tested for \textit{Chlamydia trachomatis} antigen with a rapid screening test (DiaSpot\textsuperscript{TM}, Bresta Perkasa, Indonesia). Serum was screened for \textit{Treponema pallidium} antibodies (IgG & IgM) by immunochromatographic method with Exact\textsuperscript{®} syphilis diagnostic device, USA; and \textit{Chlamydia trachomatis} antibodies (CT IgG) with ImmunoComb\textsuperscript{®}, Orgenics Ltd., Yavne, Israel.

Statistical Analysis
Data was analyzed using the statistical package of social science (SPSS) software 15.0 version. For quantitative variables, paired student’s t-test was used to test for mean differences. Pearson’s correlation analysis was employed to determine associations between variables. For non-quantitative variables, $\chi^2$-square analysis was used for determination of associations between variables. Significant $p$ was <0.05.

RESULTS
The screening tests for the afore-mentioned were all negative, suggesting that none of the patients had current genital tract infection. Chlamydial antibodies were further tested for; to identify women with previous infection which may have led to the tubal damage. Table 1 shows the comparison of Chlamydia trachomatis IgG (CT IgG) antibody positivity in Fertile and Infertile Women.

<table>
<thead>
<tr>
<th></th>
<th>Infertile (n=40)</th>
<th>Fertile (n=32)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT IgG +ve</td>
<td>Subjects 20</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>% 500</td>
<td>18.3</td>
<td></td>
</tr>
<tr>
<td>CT IgG -ve</td>
<td>Subjects 20</td>
<td>26</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>% 500</td>
<td>81.7</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Comparison of Chlamydia trachomatis IgG (CT IgG) antibody positivity in Fertile and Infertile Women

DISCUSSION
The total antioxidant potential (TAP), total plasma peroxides (TPP) and therefore, oxidative stress index (OSI) were not significantly different between infertile women and their fertile controls. This finding is in agreement with other authors, who reported no significant differences in TAP in infertile and fertile controls\textsuperscript{26,27}. Total plasma peroxides were positively associated with BMI; this indicated that increasing BMI may be a risk factor for increased oxidative stress and the associated deleterious effects on the general body system. Oxidative stress has been implicated in the pathogenesis of more than 100 disease conditions including infertility\textsuperscript{16}. The place of obesity in anovulatory infertility has been discussed—however, the subjects of the current study were ovulating, as observed by their mid-luteal progesterone assays. This finding suggests that BMI may increase oxidative stress and its influence on infertility in the absence of anovulation.

The negative correlation observed between oestradiol and waist and hip circumferences imply that obesity in women may lead to a hormonal imbalance that may reduce their fertility. It has been reported that ovulating subfertile women with a BMI over 29 kg/m\textsuperscript{2} have lower pregnancy rates compared with those with normal weight\textsuperscript{28}. Our study also points to this fact as indicated by the increased oxidative stress.
We did not expect the fertility hormone profile to be significantly different between infertile and fertile women in this study; the profile was studied to ascertain that the cases did not have anovulatory infertility, which could be a confounder. The only isolated significant hormone was luteinizing hormone. The current study cannot fully explain the association; however, the mid-luteal progesterone suggested these women were actually ovulating—therefore, the elevated LH may be of little consequence. The study design did not allow for testing mid-luteal progesterone in the controls. We required these fertile controls to be contraceptive- and hormone-naïve, so they were recruited at their first visit to family-planning clinic. A mid-luteal progesterone assay would have required a follow-up visit, but by this time, they would have been...

<table>
<thead>
<tr>
<th>Index</th>
<th>Infertile n = 40</th>
<th>Fertile n = 32</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>32.3±4.0</td>
<td>33.5±4.8</td>
<td>-1.185</td>
<td>0.240</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.2±11.4</td>
<td>66.1±14.4</td>
<td>1.019</td>
<td>0.312</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.6±0.1</td>
<td>1.6±0.1</td>
<td>0.423</td>
<td>0.673</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.1±0.4</td>
<td>26.1±5.1</td>
<td>0.879</td>
<td>0.382</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>84.9±8.3</td>
<td>84.4±12.4</td>
<td>0.179</td>
<td>0.858</td>
</tr>
<tr>
<td>HC (cm)</td>
<td>103.7±9.6</td>
<td>103.0±12.4</td>
<td>0.272</td>
<td>0.786</td>
</tr>
<tr>
<td>WHR</td>
<td>0.82±0.0</td>
<td>0.82±0.1</td>
<td>-0.027</td>
<td>0.979</td>
</tr>
<tr>
<td>TAP (μmol TE/L)</td>
<td>767.9±146.8</td>
<td>748.9±90.2</td>
<td>0.643</td>
<td>0.522</td>
</tr>
<tr>
<td>TPP</td>
<td>6.3±7.8</td>
<td>4.4±1.4</td>
<td>1.337</td>
<td>0.185</td>
</tr>
<tr>
<td>OSI</td>
<td>0.9±0.9</td>
<td>0.6±0.2</td>
<td>1.569</td>
<td>0.121</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>13.4±11.8</td>
<td>10.1±4.7</td>
<td>1.462</td>
<td>0.148</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>16.1±21.2</td>
<td>5.7±3.9</td>
<td>2.712</td>
<td>0.008</td>
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<tr>
<td>Prolactin (IU/L)</td>
<td>452.2±486.2</td>
<td>611±874.9</td>
<td>-0.976</td>
<td>0.332</td>
</tr>
<tr>
<td>P4 (pmol/L)</td>
<td>22.6±19.8</td>
<td>-</td>
<td>3.264</td>
<td>-</td>
</tr>
<tr>
<td>E2 (pmol/L)</td>
<td>0.85±1.87</td>
<td>0.51±0.41</td>
<td>1.000</td>
<td>0.321</td>
</tr>
</tbody>
</table>

*see text for explanation*

BMI- body mass index, WC-waist circumference, HC-hip circumference, WHR- waist-hip ratio, TAP- total antioxidant potential, TPP- total plasma peroxides, OSI- oxidative stress index, FSH- follicular-stimulating hormone, LH-luteinizing hormone, P4- progesterone, E2- oestrogen
commenced on a contraceptive method, so would not be suitable for inclusion any longer. The relatively high values of prolactin in the fertile women were probably because most of them were still nursing their infants at the time of recruitment. The prevalence of Chlamydia antibody positivity was found to be significantly higher in infertile women compared to their fertile controls. This finding is in agreement with the findings of other workers.3,29-34. Chlamydial infection evokes pathologic immune response and generation of reactive oxygen species which results in inflammatory response, apoptosis, tissue damage, scarring, fibrosis, hydrosalpinx, tubal occlusion leading to tubal infertility.

The findings of this study may be limited by the small sample size; the association of obesity with tubal infertility may be validated in the larger study to follow. The study will include evaluation of cellular as well as humoral pathologic response found associated with tubal infertility, and will hopefully shed more light on the aetiopathogenesis of infertility.

CONCLUSION
Previous Chlamydial infection is significantly associated with tubal factor infertility. Obesity seems to reduce fertility potential in women with tubal factor infertility as well as increase oxidative stress. Larger studies are required to explore this.

REFERENCES


