Cytokine profile in Nigerians with tubal infertility

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Abstract

Introduction: Immune response to genital Chlamydia trachomatis infection is involved in both immunity and pathology. The cytokine profile during infection has been implicated in the disease outcome, either resolution or severe sequelae. Serum cytokines of Chlamydia positive Nigerian women with tubal infertility were assessed to determine their possible relationship with tubal occlusion.

Material and methods: One hundred and fifty age-matched consenting women (100 fertile and 50 with tubal infertility) were recruited based on C. trachomatis antibody positivity and grouped into infertile Chlamydia positive (Ctpos) women (n = 50), fertile Chlamydia positive women (n = 50) and fertile Chlamydia negative (CTneg) women as controls (n = 50). High vaginal swabs and endo-cervical swabs were collected for microscopy, culture and gram staining. Cytokines [transforming growth factor β1 (TGF-β1), interferon γ (IFN-γ), tumor necrosis factor α (TNF-α), interleukin (IL)-4, IL-10 and IL-17A] were estimated by ELISA in sera. Data were analyzed using ANOVA, χ2 and Spearman’s correlation at p = 0.05.

Results: Lower IFN-γ levels were observed in infertile women compared to fertile women. Fertile CTneg women had significantly higher TNF-α, and TGF-β1 compared to fertile and infertile Ctpos women, respectively. Lower IL-10 levels were seen in fertile Ctpos women compared to the infertile Ctpos group. Vaginal discharge was negatively correlated with TNF-α and IFN-γ and positively with IL-4 in Chlamydia positive women.

Conclusions: Chlamydia positive women with tubal infertility have higher IL-10 and lower IFN-γ levels than controls, which may contribute to their development of tubal pathology.
Transforming growth factor β1 (TGF-β1) is secreted by regulatory T cells to prevent tissue damage [6]. They reduce the amount of mucosa inflammation but may contribute to bacterial persistence and colonization of the genital tract and in peritoneal adhesions [4, 7].

Deficiencies or overstimulation in the production and activity of these cytokines may be associated with failure of protective immunity or harmful inflammation. Determining the specific responses that promote tissue damage and differentiating them from those that lead to benign resolution of infection may be important in predicting the disease outcome. This study therefore examines the serum cytokine profile of Chlamydia positive Nigerian women with tubal infertility to determine their possible relationship with tubal occlusion.

Material and methods

Study design

The study was a case control study conducted in the Gynaecology and Family Planning Clinics of the Department of Obstetrics and Gynaecology, University College Hospital and Adeoyo Maternity Hospital, Ibadan, Nigeria. The study protocol was approved by the University of Ibadan/University College Hospital Ethics Committee reference number UI/EC/08/0083. This study was conducted between April 2009 and January 2010. Informed consent was obtained from the subjects before recruitment into the study.

Inclusion criteria: consenting subjects with infertility of at least one year’s duration, child birth of less than 2 years for the fertile controls. Exclusion criteria: subjects with previous history of uterine surgery, subjects undergoing any form of contraceptive therapy, those with malignancies on long-term medication, or chronic organ or systemic illness, and those that did not give consent. Infertility was defined in this study as the inability of a couple to conceive after a consecutive period of 12 months of unprotected sexual intercourse [8]. Evidence of fertility was taken as ability to have at least one child, with the last childbirth within the last 2 years. Evaluation of infertility was carried out using standard procedures according to the National Health Service evaluation criteria [9].

Socio-demographic characteristics of the study population – family history, social history, past medical history, medication and gynaecological history were obtained using a semi-structured questionnaire. Anthropometric indices – height, weight, hip and waist circumference were taken to calculate the body mass index and waist-to-hip ratio, respectively. The women were screened for the presence of Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis, Treponema pallidum, Staphylococcus aureus and Candida albicans using standard methods [10], to rule out any current reproductive tract infection, which could be a confounder. These women were further screened for presence of Chlamydia trachomatis IgG antibodies (CTIgG).

Bilateral tubal blockage was identified by hysterosalpingogram (HSG). A significant number of patients are unable to afford diagnostic laparoscopy in our resource-poor setting, therefore, HSG diagnosis was used for consistency. Other causes of tubal blockage were ruled out in those that had laparoscopy. Cases of unilateral tubal blockage were excluded, as sexually-transmitted, ascending Chlamydial infection is usually a bilateral disease. It was assumed that unilateral damage was due to other causes.

Selection of subjects

A total of 150 age-matched women of reproductive age (100 fertile and 50 with tubal infertility) without microbial antigens (Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis, Treponema pallidum, Staphylococcus aureus and Candida albicans) were consecutively recruited into this prospective case control study. These women were sub-divided into 3 groups based on chlamydia antibody positivity into infertile Chlamydia positive women (n = 50), fertile Chlamydia positive women (n = 50) and corresponding fertile Chlamydia negative women as controls (n = 50). Recruitment was discontinued after the first 50 women in each group were identified. Further data were not collected or analyzed on subsequent recruited participants in the completed groups, while other participants in other groups were still being recruited.

Sample collection

Ten milliliters of venous blood samples were collected aseptically from each subject at recruitment. Samples were dispensed into 10 ml plain sample containers. After clot retraction, samples were centrifuged at 500 g for ten minutes after which sera were extracted and stored in small aliquots at −20°C until time of analysis. High vaginal swabs (HVS) and endocervical swabs (ECS) were collected from all subjects of study for isolation of such pathogens as Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis, Treponema pallidum, Staphylococcus aureus and Candida albicans within one hour after sample collection. Cytokines (TGF-β1, IFN-γ, TNF-α, IL-4, IL-10 and IL-17A) were estimated in the sera of the study population.

Laboratory methods

Detection of Chlamydia antigens was carried out by the immunochromatographic method using prepared test kits (Diaspots Diagnostics, USA) [11]. Isolation of Candida spp. and bacterial vaginosis were done by Gram staining procedure; reagents procured from Oxoid chemicals (USA) [12]. Identification of Trichomonas vaginalis was done by microscopy [13]. Isolation of Neisseria gonorrhoeae was made by culture method using Thayer Martins culture media (Becton, Dickinson and Company, USA) [14]. Quantification of Chlamydia antibodies was carried out by the
enzyme immunoassay (EIA) method using a prepared test kit (Organics Ltd, Germany) [15]. Detection of *Treponema pallidum* antibodies was done by immunochromatographic methods with test kits (Acon diagnostics, USA) [16]. All cytokines: TGF-β1, IFN-γ, TNF-α, IL-4, IL-10 and IL-17A, were estimated by the enzyme linked immunosorbent assay (ELISA) (eBioscience, USA) [17-20].

**Statistical analysis**

Data were analysed using the statistical package for social sciences (SPSS version 20.0). Analysis of variance (ANOVA) was used to test significance of variations within and among group means. Fisher’s least significant difference (LSD) test was used for comparison of multiple group means. χ² analysis was used for comparison of means for non-quantitative variables while Spearman’s correlation was used to determine associations between non-parametric variables. A two-sided probability value *p* < 0.05 was considered statistically significant.

**Results**

The mean age, anthropometric indices [weight, height, body mass index (BMI), waist circumference (WC), hip circumference (HC), and waist–hip-ratio (WHR)] and cytokine profile of fertile women (CTneg and CTpos) and infertile *Chlamydia* positive women are shown in Table 1. Significant variations in the levels of IFN-γ were observed among the groups (*p* < 0.05). No significant variations were observed in the mean age, anthropometric indices and other indices among the groups (*p* > 0.05).

Comparison of gynecologic characteristics and symptoms of genital *Chlamydia* infection (GCI) in fertile women (CTneg and CTpos) and infertile *Chlamydia* positive women are shown in Table 2. Significant differences were observed in such characteristics as dysmenorrhea and symptoms as vaginal discharge, vaginal itching and lower abdominal pain among the groups (*p* < 0.05). No significant difference was observed in the women in relation to dyspareunia among the groups (*p* > 0.05).

Table 3 shows a comparison of serum cytokines (TGF-β1, IFN-γ, TNF-α, and IL-10) of fertile women (CTneg and CTpos) and infertile *Chlamydia* positive women using Fischer’s LSD post hoc analysis. Fertile CTneg controls had significantly higher TNF-α as compared to fertile CTpos, and also higher IFN-γ and TGF-β1 compared to infertile CTpos women (*p* < 0.05). Fertile CTpos women had significantly elevated IFN-γ and lower IL-10 compared to the infertile CTpos group (*p* < 0.05).

Table 4 shows a correlation of vaginal discharge with IFN-γ, TNF-α and IL-4 in *Chlamydia* positive women (fertile and infertile). Vaginal discharge correlated negatively with IFN-γ (*r* = –0.767, *p* = 0.005), and TNF-α (*r* = –0.430, *p* = 0.000) and positively with IL-4 (*r* = 0.253, *p* = 0.11).

### Table 1. Age, anthropometric indices and cytokines in fertile women (CTneg and CTpos) and infertile *Chlamydia* positive women (CTpos)

| Index          | Fertile CTneg *n = 50* | Fertile CTpos *n = 50* | Infertile CTpos *n = 50* | F    | p  |
|----------------|-------------------------|------------------------|--------------------------|------|----|
| Age (years)    | 34.90 ±0.46             | 33.94 ±0.48            | 33.76 ±0.51              | 1.099| 0.351|
| Weight (kg)    | 68.60 ±2.19             | 64.92 ±1.83            | 69.60 ±1.38              | 1.38 | 0.249|
| Height (cm)    | 159 ±7                  | 150 ±1                 | 160 ±1                   | 0.855| 0.465|
| BMI (kg/m²)    | 26.90 ±0.78             | 25.87±0.65             | 27.01 ±0.49              | 0.709| 0.454|
| WC (cm)        | 86.34 ±1.84             | 83.40 ±1.65            | 86.64 ±1.19              | 1.168| 0.323|
| HC (cm)        | 103.94 ±1.72            | 102.76 ±1.71           | 104.32 ±1.18             | 0.202| 0.895|
| WHR            | 0.83 ±0.07              | 0.81 ±0.07             | 0.83 ±0.06               | 1.937| 0.125|
| **Cytokines**  |                         |                        |                          |      |    |
| TGF-β1 (pg/ml) | 545.52 ±108.90          | 386.62 ±68.16          | 348.58 ±72.20            | 1.81 | 0.147|
| IFN-γ (pg/ml)  | 128.58 ±9.36            | 126.12 ±9.66           | 72.20 ±0.20              | 16.67| 0.000*|
| TNF-α (pg/ml)  | 134.61 ±25.12           | 79.94 ±2.10            | 92.77 ±19.30             | 1.68 | 0.172|
| IL-4 (pg/ml)   | 32.90 ±5.44             | 33.02 ±24.35           | 37.48 ±5.9               | 0.214| 0.887|
| IL-10 (pg/ml)  | 23.62 ±2.97             | 16.93 ±0.64            | 28.99 ±6.67              | 0.792| 0.449|
| IL-17A (pg/ml) | 37.58 ±12.75            | 28.18 ±10.04           | 43.50 ±15.53             | 0.404| 0.751|

* TGF-β1 – transforming growth factor β1; IFN-γ – interferon γ; TNF-α – tumor necrosis factor α; IL-4 – interleukin 4; IL-10 – interleukin 10; IL-17A – interleukin 17A; * – significant at *p* < 0.05; *p* – significant level; *F* – F-ratio; BMI – body mass index; WC – waist circumference; HC – Hip circumference; WHR – waist-to-hip ratio; CTpos – Chlamydia positive; CTneg – Chlamydia negative
Discussion

The cytokine milieu in genital *Chlamydia* infection has been implicated in the disease outcome; either in resolution or severe sequelae such as tubal occlusion. In this present study, fertile women (CTneg and CTpos) had significantly higher IFN-γ levels compared to *Chlamydia* positive women with tubal infertility (*p < 0.05*). This is consistent with findings of Agrawal et al. [21] who reported higher IFN-γ levels in *Chlamydia* negative and positive fertile women compared to those with fertility disorders. Women with *Chlamydia* infection without pathological damage have been shown to secrete higher amounts of IFN-γ than women who developed sequelae to *Chlamydial* infection (tubal damage) suggesting that IFN-γ is down-regulated in women with damaging sequelae [22]. Cervical cell production of IFN-γ in response to stimulation with *Chlamydia trachomatis* elementary bodies (EBs) has been positively correlated with fertility in *C. trachomatis*-seropositive individuals [23, 24]. It appears that higher levels of IFN-γ seen in fertile women with or without GCI may be responsible for their protection against tubal pathology. These observations suggest that reduced IFN-γ may be involved in the development of *Chlamydia*-induced tubal occlusion [7]. Some of the mechanisms employed by

| Table 2. Comparison of gynecologic characteristics and symptoms of GCI in fertile women (CTneg and CTpos) and infertile *Chlamydia* positive women (CTpos) |
|------------------|------------------|------------------|------------------|------------------|------------------|
| **Index**       | **Fertile CTneg** | **Fertile CTpos** | **Infertile CTpos** | **χ²** | **p** |
| Vaginal discharge | yes             | 10 (13.7%) | 18 (24.7%) | 45 (61.6%) | 53.85 | 0.000* |
|                  | no              | 40 (51.9%) | 32 (41.6%) | 5 (6.5%) | 19.27 | 0.000* |
| Vaginal itching  | yes             | 7 (9.6%)   | 16 (21.9%) | 50 (68.5%) | 6.88 | 0.032* |
|                  | no              | 43 (55.8%) | 34 (44.2%) | 0 (0.0%) | 37 (29.1%) | 0.714 |
| Dysmenorrhhea    | yes             | 6 (26.1%)  | 4 (17.4%)  | 13 (56.5%) | 0.674 | 0.714 |
|                  | no              | 44 (34.6%) | 46 (36.2%) | 3 (23.0%) | 36 (28.3%) | 0.007* |
| Dyspareunia      | yes             | 5 (38.5%)  | 5 (38.5%)  | 3 (23.0%) | 5 (6.5%) | 0.000* |
|                  | no              | 45 (32.8%) | 45 (32.8%) | 37 (29.1%) | 9 (6.5%) | 0.000* |

Values are given as the number of subjects with a percentage in parenthesis; CTpos – *Chlamydia* positive; CTneg – *Chlamydia* negative; * – significant at *p < 0.05*; GCI – genital *Chlamydia* infection

| Table 3. Comparison of cytokines in fertile women (CTneg and CTpos) and infertile *Chlamydia* positive women (CTpos) using LSD post-hoc analysis |
|------------------|------------------|------------------|------------------|------------------|------------------|
| Parameter Groups | Mean diff. | STD error | *p* |
| Fertile CTneg | 134.61 ±25.12 | 0.037 |
| Fertile CTpos | 79.94 ±2.10 | 0.046 |
| INF-γ (pg/ml) | 128.58 ±9.36 | 0.000* |
| TGF-β1 (pg/ml) | 545.52 ±108.90 | 0.033* |
| TNF-α | 7.20 ±0.20 | 0.037 |
| IL-10 (pg/ml) | 53.910* |
| INF-γ (pg/ml) | 126.12 ±9.66 | 0.000* |

Values are given as the number of subjects with a percentage in parenthesis; CTpos – *Chlamydia* positive; CTneg – *Chlamydia* negative

| Table 4. Correlation of cytokines with vaginal discharge in *Chlamydia* positive women (n = 100) |
|------------------|------------------|------------------|------------------|------------------|------------------|
| **Indices**       | **r** | **p** |
| Vaginal discharge Vs INF-γ | −0.767 | 0.005* |
| INF-γ | −0.430 | 0.000* |

*p* = significant level; TNF-α – tumor necrosis factor α; INF-γ – interferon γ; r = Spearman’s correlation coefficient; IL-4 – interleukin 4; GCI – genital *Chlamydia* infection; * – significant at *p < 0.05*
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IFN-γ to control Chlamydia replication includes promoting the engulfment and destruction of extracellular EBs [25], up-regulation of inducible nitric oxide synthase [26], induction of indoleamine-2,3-dioxygenase (IDO) activity and down-regulation of the transferrin receptor thereby depleting the intracellular stores of iron available to the organism [27].

Tumor necrosis factor α (TNF-α) is a potent pro-inflammatory cytokine that plays a crucial role in the immune response to Chlamydia trachomatis infection. TNF-α is known to have a variety of effects on host cells, including the induction of apoptosis and the stimulation of the immune response. It is also involved in the control of Chlamydia replication and the establishment of an inflammatory response.

Levels of TNF-α were also found to be significantly higher in fertile CTPOS women compared to CTNEG, compared to fertile CT Negative controls. Consistently with our findings, some studies have shown that TNF-α is a key cytokine in the immune response to Chlamydia infection. TNF-α is known to have a variety of effects on host cells, including the induction of apoptosis and the stimulation of the immune response. It is also involved in the control of Chlamydia replication and the establishment of an inflammatory response.

Chlamydia inclusion membrane protein (MOMP) is a major surface protein of Chlamydia, and it is involved in the establishment of a persistent infection. The presence of MOMP is associated with the persistence of Chlamydia infection and the development of chronic inflammatory disease. The staining intensity of TGF-β1 in the glandular epithelium and stromal cell of women with hydrosalpinges was significantly higher than those in fertile women. TGF-β1 is a cytokine that plays a crucial role in the regulation of the immune response to Chlamydia infection. TGF-β1 is known to have a variety of effects on host cells, including the induction of apoptosis and the stimulation of the immune response. It is also involved in the control of Chlamydia replication and the establishment of an inflammatory response.

The findings of this present study have shown that higher levels of IFN-γ, TNF-α and TGF-β1 in fertile Chlamydia-negative women may be responsible for their protection against Chlamydia infection. Higher levels of IL-10 and lower IFN-γ may be implicated in Chlamydia-induced tubal occlusion.

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The authors declare no conflict of interest.

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