

Serum Cytokine Concentrations in Infertile And Fertile Women. A Preliminary Study in Port Harcourt, Nigeria

Okpalaji, Cb¹, Okerengwo, Aa¹, Okpani, Aou², Chinko, Bc³, Bamigbowu, Eo⁴

¹Department Of Haematology And Blood Transfusion/ University Of Port Harcourt, Port Harcourt Nigeria

²Department Of Obstetrics And Gynaecology/ University Of Port Harcourt Teaching Hospital, Port Harcourt Nigeria

³Department Of Human Physiology/ University Of Port Harcourt, Port Harcourt Nigeria

⁴Department Of Chemical Pathology/ University Of Port Harcourt, Port Harcourt Nigeria

Abstract: The management of reproductive failure and infertility requires careful consideration of the endocrine, chromosomal, anatomic, infectious and immunological factors. Current studies have implicated the pro-inflammatory and anti-inflammatory cytokines to the success and failure of pregnancy. This study was aimed at determining the serum concentration of pro-inflammatory (TNF α , IFN γ) and anti-inflammatory (IL10, IL6) cytokines in infertile and fertile women. This is a case-control study involving 80 women (40 infertile women and 40 fertile women) attending the fertility and family planning clinics of the Obstetrics and Gynaecology Department of the University of Port Harcourt Teaching Hospital, Port Harcourt Nigeria. 3mls of their blood were collected and the serum levels of the cytokines (TNF- α , IFN- γ , IL-6, and IL-10) were estimated by Enzyme Linked Immunosorbent Assay (ELISA) and the results were compared. There was a statistically significant increase in IFN- γ (31.88 \pm 26.80) levels in the infertile women when compared with the fertile group (13.81 \pm 9.8), ($p < 0.05$). In contrast there was no statistically significant difference in the levels of IL-6, IL-10 and TNF- α between the two groups. The result of this study suggest that an increase INF- γ may be implicated in infertility.

Keywords: fertility, infertility, cytokines; IL-6, IL-10, TNF α , IFN γ .

I. Introduction

Fertility is a complex phenomenon and depends on the normal functioning of the important biological systems and mechanisms but when any of the reproductive systems fail in its functions in either the male or the female partner or both, infertility occurs. It can be classified into primary and secondary infertility. Primary infertility is defined as inability to conceive or carry a pregnancy to live birth while secondary infertility is the inability to conceive after an earlier pregnancy (1). Cytokine production is important in all the processes that will lead to pregnancy as well as maintaining the pregnancy to full term. Depending on their inflammatory reactions or responses, cytokines are broadly categorized into pro-inflammatory and anti-inflammatory cytokines produced by Th1 and Th2 cells respectively. Type1 cytokines (IL-2, IFN- γ , IL-12, IL-15, TNF- α) are those that enhance cell-mediated immunity. They support inflammation and probable loss of the pregnancy. Type2 cytokines (IL-4, IL-5, IL-6, IL-10, IL-13) are those that mainly augment humoral immunity and are anti-inflammatory and support pregnancy (2).

Recent studies suggest that a successful pregnancy is a Th2- related phenomenon and demonstrated a shift from a predominant Th1-driven cell mediated immunity to predominant Th2-driven humoral immunity in pregnant mice. (3)(4). The same report also postulate that high concentrations of type1 cytokines are associated with spontaneous abortions and fetal re-absorption in mice (4). Likewise studies in humans show that circulating levels of TNF- α and IFN- γ are higher in subjects with subsequent miscarriages compared with those with successful pregnancies (5). Also studies have shown that IFN- γ acts as growth and differentiation factor in regulating testicular and glandular functions and IFN- γ has also been shown to influence spermatogenesis (6). Other cytokines like IL-1-beta, IL-6 and TNF- α are essential in ovarian cycle regulation and play an important role during growth and development of ovarian follicle (7). In addition, increased production of TH-1 cytokines such as TNF- α and IFN- γ compared to the TH-2 cytokine IL-10 is linked to infertility and recurrent spontaneous abortion (RSA) (8). Produced by TH-1 cells, TNF- α is a multifunctional pro inflammatory cytokine secreted predominantly by monocytes/macrophages that has effects on lipid metabolism, coagulation, insulin resistance and endothelial function. Evidence was supported by previous studies in which higher serum levels of TNF- α were detected in RSA groups and reproductive failure (9). It is suggested that TH-1 cytokines trigger thrombotic/inflammatory processes at the maternal utero-placental blood vessels by activation of vascular endothelial cell pro-coagulant (10). Significantly, higher serum levels of TH-2 cytokines, IL-6 and IL-10 were detected in normal pregnancy (11). Thus, cytokine production is important to mount a type of immune response for pregnancy maintenance.

II. Materials And Methods

2.1 Research Subjects:

The study involved 40 women of child bearing age (25-40yrs) who were attending the fertility clinic and 40 non-pregnant multiparous women (25-40yrs) who were attending the family planning clinic were used as the control, both of which clinics are in the Obstetrics and Gynaecology Department of the University of Port-Harcourt Teaching Hospital (UPTH).

Parameters	Control n=40	Subject n=40	Z-test (p-value)
IL-6 (pg/ml)	3.48 ± 0.91	3.61 ± 1.36	p= 0.85
IL-10 (pg/ml)	12.66 ± 1.46	12.23 ± 1.55	p=0.10
TNF-α (pg/ml)	6.32 ± 1.28	6.45 ± 1.06	p= 0.26
INF-γ (pg/ml)	13.81 ± 9.8	31.88 ± 26.80*	p= 0.01

2.2 Inclusion Criteria

The study involved all apparently healthy women of gestational age who have not conceived at all (primary infertility) or have conceived before but cannot conceive again (secondary infertility) and apparently healthy non-pregnant multiparous women both from the fertility and family planning clinic of the same Department. Patients with chronic history of hypertension, renal disease, intrauterine growth, retroviral infection, sickle cell and malignant diseases were excluded from the study.

2.3 Ethical Consideration

Ethical permission to conduct this study was obtained from the Medical Ethics Committees of University of Port-Harcourt Teaching, Port-Harcourt, Nigeria. Informed consent forms were signed by all subjects involved in the study. All data collected including identity and results for each participant were kept confidential.

2.4 Procedure

About 3mls of venous blood was aspirated from each patient using aseptic procedures and dispensed into a plain bottle. The blood was allowed to clot at room temperature for 30 minutes and then centrifuged for about 15 minutes at approximately 1000xg to separate the cells. The resulting supernatant which is serum was transferred into a sterile polypropylene tube using a Pasteur pipette. Serum samples collected were stored at - 20°C until the test analysis was carried out. The serum concentration of IL-6/IL-10/TNF-α/INF-γ were assayed using commercially high sensitive and specific human enzyme-linked immunosorbent assay (ELISA) kits from Aviva Systems Biology, San Diego, CA, USA following the laid down protocols.

2.5 Statistical analysis:

The results were analyzed using SPSS version 20 software. The mean values for serum IL-6, IL-10, TNF-α and INF-γ were determined for the study and control groups. The mean values for the subject and the control were compared using the Z-test at p<0.05

III. Results

Table 1: Mean serum values of cytokine concentrations in infertile (subject) and fertile (control) women.

*Values are given as mean ± standard deviation *Significantly different compared to control*

There was a statistically significant increase in INF-γ (31.88 ± 26.80) levels in the infertile women when compared with the fertile group (13.81 ± 9.8), (p<0.05). In contrast there was no statistically significant difference in the levels of IL-6, IL-10 and TNF-α between the two groups. This is shown on the table below.

IV. Discussion

TH1 are pro-inflammatory cytokines (INF-γ and TNF-α) while TH2 cytokines are anti-inflammatory cytokines (IL-6 and IL-10) (12). In normal pregnancy, immune response is more of a TH2-like response which protects the foetus from being rejected (13). In this study the level of these cytokines were evaluated with the aim of comparing the serum cytokine concentrations in fertile and infertile women.

This study showed a significant increase in the level of the pro-inflammatory cytokines. For example INF-γ had a value of (31.88 ± 26.80) in the infertile women when compared with the fertile group which have a value of (13.81 ± 9.8). This finding is in agreement with the study by Raghupathy et al (14) which also showed that raised INF-γ levels are incompatible with successful pregnancy. Also a study by Wegmann et al (3) reported that raised INF-γ at implantation site is incompatible with a successful gestation. For TNF-α there are

no significant differences in the concentrations between the subjects and the control (fertile) groups. This observation contradicts that of Arck et al (15) and Clark et al (16) which showed that TNF- α has both an anti-reproductive effect and an anti-embryonic effect that trigger off foetal loss. The normalcy in our result may be due to the different status of infertility of the women studied or the different causes of the infertility in these women.

For the anti-inflammatory cytokines (IL-6 & IL-10) this study showed no significant increase in their levels in the infertile women when compared with the fertile women. This supports a previous report that TH2 cytokines supports normal pregnancy by Dalton et al (11). The present study however differ from another study by Mahdi (17) which showed an increase in IL-10 in infertile women when compared with the fertile women. Mahdi (17) suggested that this increase might have been induced by various pregnancy-specific glycoproteins (17(PSG)) that have an essential role in the regulation of maternal immune response.

V. Conclusion

This study showed a significantly elevated concentration of serum IFN- γ in the infertile women. This has demonstrated a possible association of increased IFN- γ concentration in the cause of infertility amongst women of child bearing age.

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