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# Determination 0f Follicular fluid Cytokines in Women with Polycystic Ovary Syndrome (PCOS) Attending Fertility Clinic in Southern Part of Nigeria

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**Abstract:- Polycystic ovary Syndrome (PCOS) is a heterogeneous endocrine disorder frequently used to diagnosed women attending fertility clinics in Nigeria, it is characterized by anovulation, hyperandrogenism and polycystic ovary syndrome, several factors have been associated with the pathophysiology of this disorder. The ovarian follicle contains the follicular fluid (f.f) and is the center of complex network of systemic and local signaling pathways whereby follicle grows, and oocyte acquires maturity, the Follicular fluid (f.f) contains complex mixtures of steroids, metabolites, proteins polysaccharides, small peptides, and antioxidants.**

**A prospective cross-sectional study was conducted in four selected fertility clinics in South-South part of Nigeria, the purposive sampling technique was used to recruit participants in this study. A total of 206 women were recruited, 101(49.1%) women fulfilled the Rotterdam criteria for the diagnosis of Polycystic Ovary Syndrome (PCOS), while 105 women (50.9%) without PCOS were included as control. Immunological analysis of cytokine levels was estimated using the Enzyme linked Immunosorbent Assay (ELISA). Biochemical hyper-androgenism as described as described by the Rotterdam criteria was observed in 69(68%) of women with PCOS, There was a statistically significant increase in Follicular fluid (F.F) levels of IL-6,IL-18, and TNF-alpha (8.616±1.037,50.409±6.068 and 27.014±3.252 respectively) in the PCOS group when compared to the control group (6.777±0.815 and 48.172±5.799, 22.291±2.683), however, it was observed that IL-12 levels was lower in the PCOS group (2.144±0.258) compared to the control group (2.212±0.266).The present study was aimed to investigate Cytokines levels in women with Polycystic Ovary Syndrome(PCOS) attending Fertility clinics.**

***Keywords:-*** *Cytokine, Follicular Fluid, Assisted Reproductive Treatment All correspondence to:*

## I. INTRODUCTION

To consider PCOS as an inflammatory disease, one has to study the cytokines which are natural inflammatory biomarkers. Cytokines are mainly categorized as anti-inflammatory and pro inflammatory cytokines according to their response and reaction in vivo. These cytokines are known to be produced by Th2 and Th1 cells. Th1 cytokines including IL-15, TNF-α, IFN-gamma, IL-12 and IL-2 are cytokines that encourage cell-mediated immunity and can contribute to pregnancy loss. Type 2 cytokines including IL-6, IL-5, IL-4, IL-13 and IL-10 are cytokines that mainly enhance humoral immunity, are anti-inflammatory in their actions and are known to contribute to a successful pregnancy (1). Cytokines are important protein mediators of immunity, inflammatory cell proliferation, differentiation, and fibrosis (2). These are the major biological processes underlying autoimmunity. Hence, it is not surprising that there is now convincing evidence that the dysregulation of cytokines plays an important role in the pathogenesis of autoimmunity.

Despite the existence of more than 100 cytokines, it has been shown that only a few of them namely TNF-α,IL-1β,IL-12,IL-15,IL-17 and IL-18, are consistently linked to the pathogenesis of autoimmune disease(3).these cytokines act alone or in collaboration with IL-12,IL-1β,IL-18,TNF α,IL-15 and other cytokines or chemokines to activate inflammation and from these revelation, it can be hypothesized that preventing the production or physiological action of these cytokines could assist in the treatment of some autoimmune disease such as rheumatoid arthritis. Cytokines are produced by white blood cells (WBC), mainly by macrophages in response to foreign antigens, pathogens (infection challenge) and in chronic inflammation (immunological activation). This study focuses on selected cytokines of autoimmune importance including IL-12, and IL-18. IL-12 is mainly released by dendritic cells, macrophage and monocytes after recognizing any pathogenic antigens using its toll-like receptors or other types of receptors (4), IL-12 can form physiological bridges between adaptive and innate immunity, it facilitates the production of IFN-gamma, and enhance the differentiation of Th1. Similarly, IL-18 produced mainly by phagocytic and actuated dendritic cells (5) act as an important cytokine in establishing and multiplying the proliferation of TH17 (6).

Various studies have shown that IL-18 and IL-12 are key cytokines in the development of most autoimmune disorders. This study aims to investigate the role of follicular fluid IL-12, IL-18, IL-6 and TNF-alpha profile in the development and progression of PCOS. Conversely, various studies have tried to establish the role of different biological structures in the development of infertility in women with PCOS. However, the underlying network and interaction of regulatory and anti/pro-inflammatory cytokines in the etiology of PCOS remain questionable and require in-depth studies. In other to understand the mechanism of reproductive cells inflammation in response to chronic infection or asymptomatic colonization of reproductive cells by microorganism, it seems logical to selectively recognize some biological active substance and also calculate the effect of their concentration in follicular fluid which is in direct contact with the female reproductive cells. The effect of follicular fluid which are biomarkers of folliculogenesis hold great potential for the study and treatment of PCOS and other female reproductive disorders.

The aim of this study is to compare follicular fluid and cytokines in polycystic ovary syndrome (PCOS) women undergoing infertility treatment.

## II. STUDY AREA

This study was carried out in selected fertility clinics across the southern part of Nigeria, some of the clinics were independent Private or Public organizations and were equipped with needed facilities and expertise for in-vitro fertilization treatment and PCOS diagnosis. Ethical clearance was obtained from the university of Benin Teaching Hospital Ethnic and Research committee. Consent obtained from study participation is voluntary and the confidentiality and privacy of all the participants was respected; they were assured that there was no penalty for refusal or withdrawal from participation. Information on medications, menstrual and clinical history was collected by administering a structured questionnaire, cross sectional study design was utilized in this study. The purposive sampling technique was utilized in this study, this involved identifying and selecting study participants who fit the inclusion criteria for this study and recruiting them based on their availability and willingness to participate in the study.

➢ *Study Population*

The study group was taken from patients receiving in-vitro fertilization treatment in the selected hospital spread across the Southern part of Nigeria.

➢ *Inclusion Criteria*

The inclusive criteria was based on the definition of PCOS adopted at the joint consensus meeting of the American society for Reproductive Medicine and the European Society of Human Reproduction and Embryology (ASRM/ESHRE) criteria.

1.Oligo-and /or anovulation
2.Hyperandrogenism (clinical and /or biochemical) and
3.Polycystic ovaries with the exclusion of other etiologies. (Rotterdam ESHRE/ASRM,2004).

➢ *Exclusion Criteria*

This study excluded women who has any of the following condition:

- History of chronic hypertension
- Known autoimmune disorder
- Women who did not give constant
- Diabetes mellitus or treatment with oral glucocorticoids
- Congenital adrenal hyperplasia.

➢ *Sample Size Determination*

The sample size was calculated using the Cochran formula for sample size determination. fertility treatment.

➢ *Follicular Fluid Sample Collection*

Follicular fluid samples were collected from the first punctured follicular during oocyte retrieval after ovarian hyper stimulation with the short antagonist protocol. Ovulation was triggered by recombinant human chorionic gonadotropin (Pregnyl, Baxter USA) when at least three follicles measured more than 17 mm. Oocytes were retrieved 36hrs later aided by trans-vaginal ultrasound guidance. Aspirated follicular fluid were aseptically transferred into a sterile plate for oocyte retrieval, the residual follicular fluid was aseptically transferred into sterile 15 ml Falcon tubes for culture and then storage at -$20^0$C for immunological assay (7)

➢ *Quantitative Estimation of Follicular Fluid Cytokines*

Follicular fluid cytokines (IL-6, IL-12, IL-18, and TNF-alpha) levels were measured using (Abcam, Massachusetts USA) ELISA kits. Briefly, Follicular fluid (FF) samples and standards were added to wells in a 96 well plate coated with monoclonal antibody specific for each cytokine, Biotinylated labeled antibody specific for each cytokine were added and incubated, the wells were washed thoroughly and the enzyme, streptavidin horseradish peroxide was added to the well and incubated, after the final wash, TMB substrate solution was added for a color reaction, the intensity of this colored products were read at 450 nm using the BioTek 800 TS Absorbance plate reader.

## III. RESULTS

A total of 206 women attending fertility clinics in 4 facilities across the study area were recruited for this study and the study subjects were divided into two groups according to the Rotterdam diagnostic criteria for PCOS. 101(49%) women who met the Rotterdam criteria for diagnosis of PCOS were grouped as the PCOS group while, 106 (51%) apparently healthy women whose partners had male factor infertility were recruited and grouped as the control group. The baseline demographic characteristics of the study of population is shown in Table 1. The study group age ranged from 26 to 53 yrs. and there was no significant difference between the mean ages of the PCOS and the control group with mean ± SD which was 29.46±4.3 for PCOS group vs 30.44±3.1 for the control group. The mean body mass index (BMI)in the PCOS (25.37±5.9). group were significantly higher than mean BMI of the control group (24.29±3.8) on the other hand, the mean number of menstrual cycle/yrs. in the PCOS group (5±4.63)

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were significantly lower than that of the control group (5±4.63 and 9±3.04).

The comparative analysis of follicular fluid cytokines in women with PCOS and the control group using Chi-square test is shown in table 2. When compared with women in the control group, women with PCOS had significantly higher follicular fluid cytokine levels of IL-6, IL-18, and TNFα. In contrast, results revealed that IL-12 levels were significantly lower in the PCOS group when compared to the control group (2.144±0.258 vs 2.212±0.266, P<0.05). Furthermore, TNFα showed a very high significant difference (P<0.001).

**Table 1: Demographic characteristics of study subjects**

| | PCOS (Mean ± SD) | Control (Mean ± SD) | P value |
|---|---|---|---|
| Mean age (years) | 29.46±4.3 | 30.44±3.1 | 0.124 |
| Number of menstrual cycle/year | 5±4.63 | 9±3.04 | 0.001* |
| Body Mass index (kg/m²) | 25.37±5.9 | 24.29±3.8 | 0.021* |

Values are mean± standard deviation (n=206),* p< 0.05 = significant

**Table 2: Follicular fluid cytokines levels in the study population**.

| Cytokine | PCOS (Mean ± SEM (pg/ml)) | Control (Mean ±SEM (pg/ml)) | p-value |
|---|---|---|---|
| IL-6 | 8.616±1.037 | 6.777±0.815 | 0.010 |
| IL-12 | 2.144±0.258 | 2.212±0.266 | 0.046 |
| IL-18 | 50.409±6.068 | 48.172±5.799 | 0.025 |
| TNFα | 27.014±3.252 | 22.291±2.683 | 0.000 |

## IV. DISCUSSION

This study focuses on investigating follicular fluid cytokine patterns in PCOS. Cytokines of importance in this study include IL-6, IL-12, IL-18 and TNF alpha. In this study, we confirm the association between these four follicular fluid cytokines IL-6, IL-12, IL-18 and TNF - alpha) and PCOS. Although all the follicular fluid cytokines investigated in this study showed statistical significance in the PCOS group when compared to the control group, some specific cytokines had higher statistical significance than others. Firstly, for IL-18, results shows a high statistical difference in mean follicular fluid levels of IL-18 in PCOS compared to the control group, findings in this study agrees with that of who measured serum IL-18 in women with different forms of infertility and reported a marked increase in IL-18 levels in the cervical secretions of women, nonetheless the study reported no strong correlation between altered IL-18 levels and fertility treatment outcomes, however, in this study, IL-18 was detected in considerably higher concentration within follicular fluid from women with PCOS higher than that measured in the control group.

IL-6 is an important discriminatory cytokine investigated among women with PCOS in this study, IL-6 is mainly a pro-inflammatory cytokine which can induce inflammatory reactions in response to infections by microorganism and can also contribute to transition of inflammatory response to low grade chronic inflammation which is often observed in most autoimmune disorders, IL-6 was observed to be significantly (p<0.05) elevated in the follicular fluid of women with PCOS in this study compared to control group.

This study reports IL-12 as a key cytokine in the development of inflammatory response in PCOS; there was a marked decrease in the follicular fluid levels of IL-12 in women with PCOS when compared to the control group of apparently fertile women. To investigate whether follicular fluid excess of some specific hormones can be associated with the altered levels of IL-12, statistical graphs revealed a negative correlation between IL-12 and testosterone which is a discriminatory androgen found in excess in the serum of women with PCOS, findings in this study is in line with previous reports which suggests that the production of certain cytokines including IL-12 may be down-regulated or altered by the excess secretion of some certain hormones like testosterone found in excess in women with PCOS this may explain the lowered levels of IL-12 observed in this study. IL-12 has been shown in previous studies to also play a regulatory role in the process of follicular development (8). However, the immuno-regulatory role of IL-12 in follicular development, oocyte fertilization and progression of pregnancy are still under research. Somigliana and colleagues (9) observed that IL-12 induces cell mediated immune reactions by NK cells targeting antigens in the endometrium, this in turn results in an improved prevention of ectopic implantation in the endometrium; Naz and Evans (10) reported a statistically significant association between semen IL-12 levels and the sperm morphology in humans. Also, Coskun and his counterparts (11) demonstrated lower levels of follicular fluid IL-12 in developing follicles just before ovulation when compared primordial early follicular phase follicles. On the other hand, (12) reported a significant correlation between follicular fluid IL-12 levels and negative fertilization outcomes following assisted reproduction techniques. It is worthy of note that few of these studies

reported any significant association between IL-12 follicular fluid levels and pregnancy outcome. This study however, observed that IL-12 was significantly lower in women with negative pregnancy outcomes. Findings in this study may suggest that IL-12, as previously observed with certain pro inflammatory cytokines (13), may interact with biological structures in a complex communicative network, to coordinate and control folliculogenesis in women with PCOS. The observations made in this study regarding IL-12 is that lowered IL-12 levels in PCOS and in women with negative pregnancy outcome may not be unrelated, since, firstly, a negative correlation was observed between follicular fluid testosterone and IL-12 levels. Whereas positive correlations were observed between follicular fluid testosterone and IL-6. Furthermore, the altered levels of cytokines reported in this study in women with PCOS may be responsible for the altered processes that usually encourage normal follicular development which is required for successful fertilization and pregnancy. Altered cytokine profiles have been reported previously in some studies that studied ovarian tissue from women with early ovarian failure, endometriosis, and ovarian cell malignancy. This study focuses on follicular fluid cytokine patterns in women with PCOS. PCOS is an endocrine disorder, presenting with androgen excess and hormonal imbalance, these extreme hormonal changes may affect immune response with altered humoral responsiveness resulting in autoimmunity. Elevated IL-6 and IL-18 as revealed from findings in this study also confirms that there is low grade chronic inflammation in the ovarian tissue of women with PCOS resulting in the development of multiple cysts and follicular growth arrest typically seen in PCOS. This study reports low concentrations of follicular fluid IL-12, this agrees with some previous studies, though these studies utilized serum instead of follicular fluid. (14), some other studies reported little or no difference in cytokine concentrations between women with PCOS and controls (15). However, this study was unable to find a strong statistical correlation between follicular fluid IL-12 levels and pregnancy outcomes.

## V. CONCLUSION

This study identified cytokines as inflammatory Biomarker in Polycystic Ovary Syndrome (PCOS) and provides evidence that dysregulation of cytokines plays an important role in the pathogenesis of PCOS. These findings are valuable addition to the huge knowledge gap on the pathophysiology of PCOS and the effects of cytokines on the post-IVF outcomes. Further characterization of cytokines present in the follicular fluid will increase our understanding of their effects on pregnancy rate and early pregnancy events.

## REFERENCES

[1]. Reid, G., Brigidi, P., Burton,J.P., Contractor, N., Duncan, S., Fargier, E., Hill, C., Lebeer, S.,Martin, R., McBain, A.J., Mor,G., O'Neill, C., Rodriguez, J.M., Swann, J., Van Hemert, S and Ansell, J. (2015). Microbes are central to human reproduction. American Journal of Reproductive Immunology.73:1-11

[2]. Viniker,D.A (1999).Hypothesis on the role of sub-clinical bacteria of the endometrium (bacteria endometriosis) in gynecological and obstetric enigmas. Human Reproduction Update 5:373-385

[3]. Dohle, G.R., (2003). Inflammatory associated obstruction of the male reproductive tract. Andrology and obstetrics 35:(5):321-4

[4]. Langrish, C.L., McKenzie, B.S., Wilson, N.J., de Waal, M.R., Kastelein, R.A and Cua, D.J(2004).IL-12 and IL-23: Master regulators of innate and adaptive immunity. Immunological Reviews 202(1):96-105.

[5]. D'Elios, M.M., Del Prete, G and Amedei, A (2010). Targeting IL-23 in human diseases. Expert Opinion on Therapeutic Targets 14(7):759-774

[6]. Hirahara, H., Ghoreschi, K., Laurence, A., Yang, X.P., Kanno Y and O'Shea, J.J., (2010). Signal transduction pathways and transcription regulation in Th17 cell differentiation. Cytokine and Growth factor Reviews 21(6):425-434.

[7]. Wissing, M.L., Bjerge, M.R., Olesen, A.G., Hoest, T. and Mikkelsen, A.L. (2014). Impact of PCOS on early embryo cleavage kinetics. Reproductive Biomedicine 28:508-514

[8]. Naz, R. and Butler, A. (1996). Interleukin-6 and 8 levels in sera and cervical mucus of fertile, idiopathic infertile and immunoinfertile women: implication in infertility. American Journal of Reproductive Immunology 35:534-540.

[9]. Somigliana, E., Vigano, P., Rossi G., garnelli, S., Vignali, M and Panina-bordignon, P. (1999). Endometrial ability to implant in ectopic sites can be prevented by interleukin-12 in a murine model of endometriosis. Human Reproduction 3:123-131.

[10]. Naz, R.K and Evans, L. (1998). Presence and modulation of interleukin-12 in seminal fluid plasma of fertile and infertile men. Journal of Andrology 19:302-307.

[11]. Coskum, S., Uzumcu, M., Jaroudi, K., Hollanders, J.M., Parhar, R.S., and Sedairy, S.T., (1998).Presence of leukemia inhibitory factor and interleukin-12 in human follicular fluid during follicular growth. American Journal of Reproductive Immunology 40:13-18

[12]. Gazvani, M.R., Bates M., Vince, G., Christmas, S., Lewis-Jones, D.I., and Kingsland, C. (2000). Follicular fluid concentration of interleukin-12 and interleukin-8 in IVF cycles. Fertility and Sterility 74:953-958

[13]. Gautam A. (2010). Complications of IVF. Journal of obstetrics and Gynecology India 60(4):297- 298.

[14]. Pelzer, E.S., Allan, J.A., Waterhouse, M.A., Ross, T.W., Beagley, K.W., and Knox, C.L., (2013). Microorganisms within human follicular environment: effects on IVF. Journal of Human Reproduction 8:59-62

[15]. Jasper, M and Norman, R, J (1995) Immunoactive interleukin-1 beta and tumor necrosis factor alpha in thecal, stromal, and granulose cell cultures from normal and Polycystic Ovaries. Human Reproduction 10:1352-1354