EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 3
79-83
Year: 2019

# ASSESSEMENT OF SOME CYTOKINES IN PREGNANCY LOSS

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**Article Received on 31/12/2018** **Article Revised on 21/01/2019** **Article Accepted on 11/02/2019**

## ABSTACT

**Background**: Human pregnancy is mediated by complex immunological alterations. **Objective**: Assessment of some cytokines in pregnancy loss in Abia State, South East, Nigeria. **Materials and Methods**: This was a cross-sectional study involving women in their reproductive years. Study population was stratified into 3 groups. The serum concentrations of the following cytokines: IFN-γ, IL-12 (Th1 cytokines), IL-10 and IL-4 (Th2 cytokines), were measured using the ELISA technique and compared among the three groups. **Results**: A total of 130 apparently healthy Nigerian women of child-bearing age were enrolled in the study. The study groups consisted of 70 women who had just lost a pregnancy, 30 women with normally progressing pregnancy and 30 nonpregnant women. Results show that the IFN-γ, IL-12 (Th1cytokines) and IL-4 (Th2 cytokines) concentrations of the pregnancy-loss subjects were significantly higher than that of the control groups. **Conclusion:** Pregnancy loss is associated with Th1 bias.

**KEYWORDS:** Pregnancy loss, cytokines.

## INTRODUCTION

Pregnancy loss (also termed miscarriage or abortion), is the spontaneous demise of pregnancy already confirmed positive by the presence of β-human chorionic gonadotrophin (β-hCG) in the urine or serum of patient (Kolte *et al.*, 2014). Spontaneous pregnancy loss is a common occurrence affecting approximately 15% of all clinically recognized pregnancies (Ford and Schust, 2009). It is the most common complication of early pregnancy (National Collaborating Centre for Women's and Children's Health (UK), 2012). It can take place at any stage of pregnancy but the vast majority of pregnancy losses take place in the first trimester (Williams, 2012). Recurrent pregnancy loss (RPL) or recurrent spontaneous abortion (RSA), also referred to as habitual abortion or recurrent miscarriage (Ford and Schust, 2009) is defined as three or more consecutive pregnancy losses prior to the 20$^{th}$ week of gestation (Szekere-Bartho and Balasch, 2008; Shankakumar *et al.*, 2011). Approximately 1-2% of couples have three or more consecutive losses (Ford and Schust, 2009). The causes of pregnancy loss and particularly of the recurrent cases are often unclear or controversial and in many instances multifactorial (Shankakumar *et al.*, 2011). Inspite of the documentad known causes and despite a wide range of investigations, up to 45-50% of miscarriages have no apparent cause (Hill, 2004; Stephenson and Kuteh, 2007; Szekeres-Bartho and Balasch, 2008). Most researchers appear to agree that a substantial proportion of these otherwise unexplained recurrent abortions may have an immunologic aetiology (Shankakumar *et al.*, 2011; Beaman *et al.*, 2012).

Cytokines are soluble regulatory proteins or glycoproteins that are produced by leucocytes and in many cases also by other cells (Calleja-Agius and Brincat, 2008) which exert complex regulatory influence on inflammation and immunity (Gulati *et al.*, 2016). This they do by acting as key messengers for and between immune cells, thus aiding chemical communication between cells and helping to maintain an intricate and delicate balance in the immune system. Cytokines are generally categorized on the basis of the cell from which they are produced either from T helper 1 (Th1) cells or T helper 2 (Th2) cells (Gulati *et al.*, 2016). Cytokine networks help to maintain homeostasis during pregnancy (Velez *et al.*, 2008). Strong inflammatory response mediated by cytokines and chemokines are also involved in pregnancy complications (Velez *et al.*, 2008). While T helper type 2 immunity to trophoblast antigens is associated with pregnancy success, Th1-type immunity is associated with unsuccessful pregnancy especially recurrent miscarriages (Wang *et al.*, 2002; Calleja-Agius and Brincat, 2008). Zenclussen *et al*, (2001), Daher *et al*, (2004) and Yu *et al*, (2005) all reported Th1 bias in miscarriages. Bates *et al*, (2002), do not support of a Th1

bias in pregnancy loss while Whitcomb *et al*, (2008), failed to observe any consistent association between cytokines and miscarriages.

## MATERIALS AND METHODS

A total of 130 apparently healthy women of childbearing age (18-45 years) were enrolled in this study, using a cross-sectional study design stratified into three groups of study: as follows: pregnancy-loss (70 subjects), normal pregnancy (30 subjects) and non-pregnant control (30 subjects). Study lasted the period between December 2017 and March 2018.

### Study Setting

The study took place at the following hospitals: Federal Medical Centre, Umuahia, Nazareth Specialist Hospital Aba, (a specialist gynaecological clinic), General Hospital, Aba and General Hospital, Ohafia, all in Abia state, Nigeria.

### Laboratory Analysis

The manufacturers' standard operation procedure (S.O.P.) for each investigation was used during each assay, and the operational instruction for each machine was strictly followed.

### Blood collection

3mls of the blood was put into plain vacuum containers and the serum retracted after 30 minutes of clotting. The serum was used for the following cytokine estimations - IFN-γ, IL-12, IL-10 and IL-4.

### Ethical Approval

Ethical approval was obtained from the Ethics Committee of the Federal Medical Centre, Umuahia, the Hospitals Management Board, Umuahia and the Nazareth Specialist Hospital, Aba.

The cytokine estimations were done using the ELISA technique with kit procured from Melsin Laboratories, China).

### Statistical Analysis

Statistical analysis was done using SPSS windows version 20 (IBM Corporation, 2011). Data was grouped into pregnancy-loss, normal pregnancy and non-pregnant control subjects. The Kolmogirov-Smirnov test was used to determine normality. Parametric data were expressed as +/- SD. Immunological and haematologic parameters were skewed. Hence, they were expressed as median, $2.5^{th}$ (p2.5) and $97.5^{th}$ (p97.5) percentile, and were log-transformed prior to analysis. ANOVA. Data was considered significant at error probability *P*-level less than or equal to (≤) .05.

## RESULTS

**Table 1: Comparison of Cytokine Concentrations between Pregnancy-Loss, Normal Pregnancy, and Non-pregnant Subjects.**

| Parameter | | Pregnancy Loss (n=70) | Normal Pregnancy (n=30) | Non-pregnant (n=30) | *F* | *p* |
|---|---|---|---|---|---|---|
| | | | | | | |
| IFN-γ (pg/ml) | *Mdn* | 175.90 | 71.40 | 110.85 | 29.08 | .00** |
| | P2.5 – P97.5 | 123.33 – 210.88 | 21.00 – 71.40 | 58.60 – 110.85 | | |
| | | | | | | |
| IL-12 (ng/l) | *Mdn* | 6.80 | 2.20 | 2.10 | 70.87 | .00** |
| | P2.5 – P97.5 | 5.96 – 8.00 | 1.30 – 2.20 | 1.40 – 2.10 | | |
| | | | | | | |
| IL-10 (ng/l) | *Mdn* | 24.20 | 22.65 | 23.15 | 2.87 | .06 |
| | P2.5 – P97.5 | 17.49 – 50.02 | 2.60 – 22.65 | 16.40 – 23.15 | | |
| | | | | | | |
| IL-4 (ng/l) | *Mdn* | 15.20 | 7.50 | 20.05 | 3.05 | .05* |
| | P2.5 – P97.5 | 0.28 – 98.86 | 2.0 – 7.50 | 0.30 – 20.05 | | |
| | | | | | | |

*Key*: n = number of subjects, *Mdn* = median, P2.5 = $2.5^{th}$ percentile, P97.5 = $97.5^{th}$ percentile, *F* = F-test statistic, *p* = error probability, IFN-γ = Interferon-gamma, IL-12 = Interleukin 12, IL-10 = Interleukin 10, IL-4 = Interleukin 4, *Significant difference observed at $p \leq .05$, **Significant difference observed at $p \leq .01$, using ANOVA.

**Table 2: Logistic Regression on the Relationship between Cytokine Concentrations and Pregnancy Loss.**

| | 95% CI for exp b | | | |
|---|---|---|---|---|
| | *B(SE)* | Lower | exp b | Upper |
| Included | | | | |
| Constant | 8.07(2.14)** | | 3203.18 | |
| IFN-γ | -0.03(0.01)*** | 0.96 | 0.98 | 0.99 |
| IL-12 | -0.85(0.19)*** | 0.29 | 0.43 | 0.63 |
| IL-10 | -0.03(0.04) | 0.90 | 0.97 | 1.04 |
| IL-4 | 0.02(0.02) | 0.99 | 1.02 | 1.05 |

*Key*: exp b = change in odds per unit change in predictor, *B* = coefficients of predictors, SE = standard error, 95% CI = 95% Confidence Interval, $R^2$ = .45 (Cox & Snell), .63 (Nagelkerke), Model $X^2(4)$ = 59.30, $p < .001$. **$p = .001$, ***$p < .001$.

**DISCUSSION**

Human reproduction is governed by complex immunological interactions between the foetal tissue and the receptive uterus (Saito, 2000). Immune dysfunction has been implicated in the pathogenesis of early pregnancy loss (Beaman *et al*., 2012) and this dysfunction may involve defects in cytokines, growth factors and immune-suppressive factors at the maternal-foetal interface (Royal College of Obstetricians and Gynaecologists, 2003). Currently, the challenge of pregnancy loss has created a growing research field especially regarding the role of cytokines in recurrent miscarriage. In this study we assessed some of these cytokines in 130 women that were shared into three groups: those who just lost a pregnancy (the study group), healthy pregnant women without history of pregnancy loss (the positive control group), and healthy nonpregnant women (who formed the negative control group). The cytokines were grouped into Th1 (IFN-γ and IL-12) and Th2 (IL-4 and IL-10).

Result shows there was significant difference in the IFN-γ concentration between the pregnancy-loss subjects (*Mdn*: 175.90 pg/ml, p2.5 – p97.5: 123.33 – 210.88 pg/ml) and normal pregnancy subjects (*Mdn*: 71.40 pg/ml, p2.5 – p97.5: 21.00 – 71.40 pg/ml, $p = .00$) (Table 1). This agrees with the work of Makhseed *et al*, (2001) and Sykes *et al*., (2012b). But the finding contrasts with that of Sehmsdoorf *et al*, (2004) who found no significant difference between RPL and normal pregnancy groups. The IFN-γ concentration of the pregnancy-loss subjects was also significantly higher than that of the non-pregnant control group (*Mdn*: 110.85 pg/ml, p2.5 – p97.5: 58.60 – 110.85 pg/ml, $p = .00$) (Table 1).

There was also significant difference in the IL-12 concentrations between the pregnancy-loss subjects (*Mdn*: 6.80 ng/l, p2.5 – p97.5: 5.96 – 8.00 ng/l) and normal pregnancy subjects (*Mdn*: 2.20 ng/l, p2.5 – p97.5: 1.30 – 2.20 ng/l) at p = .00 (Table 1). Similarly the IL-12 concentration of pregnancy-loss subjects was significantly higher than that of the nonregnant control subjects (*Mdn*: 2.10 ng/l, p2.5 – p97.5: 1.40 – 2.10, p = .00). Both IFN-γ and IL-12 are Th- type 1 cytokines which are reported to be significantly raised in miscarriages, particularly recurrent types (Zenclussen *et al*., 2001; Daher *et al*., 2004; Yu *et al*., 2005; Comba *et al*., 2015).

The Th type-2 cytokines studied in this work are IL-4 and IL-10. We observed significant increase in IL-4 concentration in pregnancy-loss subjects (*Mdn*: 15.20 ng/l, p2.5 – p97.5: 0.28 – 98.86 ng/l) when compared with concentrations in normal pregnancy subjects (*Mdn*: 7.50ng/l, p2.5 – p97.5: 2.0 – 7.50 ng/l) at $p = .05$ (Table 1). This contrasts with the findings by Abdullah and Mahdi (2013) who found nonsignificant difference in IL-4 concentrations between women with spontaneous abortion and normal pregnant controls. But our result agrees with the result by Bates *et al*, (2002) who found raised IL-4 levels in pregnancy-loss subjects (indicating an accentuation, rather than a shift in Th1/Th2 balance when pregnancy-loss subjects were compared with normal pregnancy subjects). This, they considered an aberration. But in our work also, IL-10) showed no significant difference across all groups ($p = .06$) (Table 1).

Genetics and differences in ethnicity could probably have contributed to variations in detected levels of cytokines between our work and other works. Miller and Cappuccio (2007) demonstrated the existence of ethnic differences in the levels of circulating inflammatory markers which they admit may be partially related to demography, lifestyle or genetic factors. Lyn-Cook *et al*, (2014) in their work on female SLE patients reported that African-American women had significantly higher levels of cytokines than their age-matched European-American counterparts. In similar vein, Velez*et al*, (2008), reported that both *in vitro* and *in vivo* findings suggest that inflammatory responses mediated by cytokines and chemokines may not be the same in different geographic populations. They considered genetic predisposition as a possible cause of individual cytokine concentration pattern. In addition they proposed a possible role of infections in African-Americans as partly accounting for the differences in cytokine expressions between Caucassians and African-Americans. However, in a limited sample study, Walston and Zellers (2014) found no such racial implications in cytokine expression levels between African-Americans and Caucassians who were on radiotherapy for breast cancer.

In their work, Sehmsdoorf *et al*, (2004), found no difference in the Th1 (TNF-α and IFN-γ), Th2 (IL-4 and IL-10) or Th3 (TGF-β2) serum levels between spontaneous abortion group and normal pregnancy group. They implicated a rapid turnover of cytokines in serum of patients studied as a possible explanation for the lack of differences in serum concentrations of cytokines between women with pregnancy loss and those with normal pregnancy. Bates *et al*, (2002), in fact found a shift towards a type 2 cytokine production in RPL as they found significant lower IFN-γ levels and significant higher IL-10 and IL-4 levels respectively in women who subsequently miscarried compared to those whose pregnancy progressed to a successful end. Thus the cytokine shift which appears to characterize normal pregnancy was accentuated, rather than diminished in pregnant women with RPL. Mahdi (2011), showed increased IL-10 (contrary to or work) and increased of IFN-γ (similar to our work) as supportive of pregnancy failure. Differences in methodologies (Kruse *et al*., 2000; Sehmsdoorf *et al*, (2004) and timing of sample collection (Kruse *et al*., 2000; Whitcomb *et al*., 2008) have equally been proposed as strong probable explanations for the lack of consistent association between cytokines and pregnancies/miscarriages. Added to these are also issues of patient selection, ethnicity, HLA incompatibility, as

well as small number of patients studied and presence of pregnancy-specific glycoproteins that induce the secretion of specific cytokines (Mahdi, 2011).

## CONCLUSION

Pregnancy loss is characterized by profound immune changes. Some of these changes are critical to pregnancy success, of which immune-related cytokines are key players. Findings from this study showed a significant association between the Th 1-type cytokines (IFN-$\gamma$ and IL-12) and first trimester pregnancy loss. That women with pregnancy loss have a significant increase in IFN-$\gamma$ and IL-12 suggests a role for these two cytokines in early pregnancy. Although clinical studies on immune-mediated pregnancy loss can be difficult to interpret, it is heart-warming to note that pregnancy success does not appear to require an intact immune system.

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