

Evaluation of Differential Levels of Serum Interleukin-6 in Pre-Eclamptic and Normal Pregnancy Women

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Abstract

There has been an increase incidence of Pre eclampsia which is one of the important causes of maternal as well as foetal mortality and morbidity. The changing life style and various stresses in this fast growing world further increase the incidence of Pre eclampsia. The exact cause is still not known beside exploratory study but it seems a state of inflammatory and oxidative stress. Our aim was to evaluate the role of Interleukin 6 (IL6) and to compare its value in normal pregnancy and pre eclampsia. We found in this study that level of IL6 is significantly high in pre-eclamptic compare to normal pregnant women. Our study shows that Pre-eclampsia pregnancy is associated with an enhanced maternal inflammatory condition.

Key words- Pre eclampsia, Interleukin-6, Inflammatory, Oxidative stress, mortality, Pregnancy

Introduction

Pre-eclampsia(PE) is pregnancy-induced hypertension (PIH) of unknown etiology, a life threatening complication of pregnancy that is characterized by new onset of hypertension and proteinuria after 20 weeks of gestation. A clinical diagnosis of PE is confirmed by chart review using the newest guidelines, published in Pregnancy Hypertension ^[1](i.e., ≥ 140 mmHg systolic blood pressure and/or ≥ 90 mmHg diastolic blood pressure with new onset proteinuria or one or more adverse/severe conditions indicated by the new guidelines).

Preeclampsia is a significant public health problem in both developed and developing countries, causing maternal and perinatal morbidity and mortality globally. The prevalence of preeclampsia varies in different populations and in different ethnic groups ^[2].

Preeclampsia occurs in 5–8% of pregnancies worldwide, and is the second leading cause of direct

maternal and foetal deaths ^[3]. Till date there is neither adianagnostic test nor screening tool available for early identification of women at risk of preeclampsia. In the developing world, severe forms of preeclampsia and eclampsia are more common, ranging from a low of 4% of all deliveries to as high as 18% ^[4]. Around ten million women develop preeclampsia each year around the world and worldwide about 76,000 pregnant women die each year from preeclampsia and related hypertensive disorders and the number of babies who die from this disorders is around 500,000 per annum ^[5].

In India, a national wise cross sectional study was done by Sutapa Agrawal and Gagandeep k walia for Prevalence and risk factors for Pre-eclampsia in Indian women. They found the highest incidence in Tripura state 87.5% and lowest in Haryana 33%. Over all pregnancy-induced hypertension (PIH) accounts for about 50000 deaths per year ^[6]. Perinatal mortality is very high (5- fold) in hypertensive disorders during pregnancy and etiology is reduced uteroplacental circulation and remains one of the most common reasons for women to die during pregnancy ^[7].

The exact cause of pre-eclampsia is still not clearly understood, despite many attempts to identify possible causes. It is proposed that multiple factors are involved in the initiation and progression of preeclampsia,

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including maternal constitutional factors, inflammatory activation, endothelial dysfunction, anti-angiogenic factors^[8]. In this study we see the role of Interleukin-6 in pre-eclampsia patients and compare the level of same in normal pregnancy and Pre-eclampsia.

Interleukin 6 (IL6) is a multifunctional pro and anti-inflammatory cytokine^[9] that was first reported in 1986^[10] and plays important roles in acute and chronic inflammation and autoimmunity^[11] and can modulate both pro and anti-inflammatory events^[9]. IL6 is widely expressed in the gestational tissues and in female reproductive tract and regulates functions in embryo implantation and placental development as well as the immune adaptations required to tolerate pregnancy. It seems that IL-6 is the very important and useful circulating marker of endothelial dysfunction^[12]. Among the inflammatory markers that are increased in preeclampsia, Interleukin 6 has been consistently indicated to be present at higher serum concentrations in pre-eclamptic patients than in normal pregnant women^[13,14]. Therefore we selected Interleukin 6 as the marker of inflammation in our study.

Material and Method

A. Selection of Cases And Control

The study was conducted on thirty normotensive third trimester pregnant women (control) and thirty newly diagnosed cases of pre-eclampsia in third trimester pregnant women. Both control and cases were selected from Obstetrics and Gynaecology out-patient and antenatal clinic of J.N. Medical College Hospital, A.M.U., Aligarh between December 2014 and October 2016 having no complications like any systemic diseases (diabetes, essential hypertension, chronic renal failure, thyroid disorders, genital tract diseases, urinary tract infection and cervical or vaginal inflammation), since these conditions cause to oxidative stress and inflammation. Informed and written consent was taken from the cases and controls for participation in the study with approval of institutional Ethical Committee, J.N. Medical College Hospital, Aligarh.

Sample Collection

Antecubital venous blood (5ml) was collected from the subjects in aseptic precautions. The blood was allowed to be kept at 2 to 8 degree Celsius in a refrigerator for one hour. After this serum was obtained by centrifugation at 3000 revolve/minute for 5 minutes. Fresh serum was

used for analysis of IL-6. The blood sample was collected in plain vials for serum Interleukin-6 estimation. All the samples were centrifuged in the PG. Lab of physiology Dept. JNMCH AMU and serum was separated for study and stored in deep freezer.

Serum IL-6 Estimation

Serum IL-6 estimation was done with the help of Human IL-6 ELISA kit as described in the user manual. The ELISA kit for IL6 was supplied by Diaclone Research, Cedex, France.

PRINCIPLE OF IL-6 ESTIMATION AND PRODUCT DESCRIPTION

The Diaclone Human IL-6 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human IL-6 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human IL6 coated on a 96-well plate. Standards and samples were pipetted into the wells and IL6 present in a sample got bound to the wells by the immobilized antibody. The wells were washed and biotinylated anti-human IL6 antibody was added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin was pipetted to the wells. The wells were again washed, a TMB substrate solution was added to the wells and color developed in proportion to the amount of IL6 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color was measured at 450 nm by the Elisareader.

Statistical Analysis of Data

Results were analysed using appropriate statistical tests with the help of Graph Pad Prism software.

- Mean
- Standard Deviation (S.D.)
- Un paired t-test
- P value.

Observation and Result

The present study was done to know the level of Serum interleukin 6 in pre-eclamptic and normal pregnant women and then these values were compared between these two groups.

Serum IL-6 levels were observed in normal pregnant women and pre-eclampsia patients (Table-1). Mean IL-6 levels in normal pregnant women were found 2.447 ± 0.786 pg/ml (N=30) and in pre-eclamptic patients were found 7.456 ± 2.647 pg/ml (N=30). This increase in pre-eclampsia patients was found highly significant (P value < 0.01).

Table-1: Mean levels of serum IL6 in normal pregnant women and pre-eclampsia patients

Mean level of serum Interleukin-6(ng/ml)	Normal Pregnant (N = 30)	Pre-eclampsia (N = 30)	P value
	2.447 ± 0.786	$7.457 \pm 2.647^*$	<0.05

*P value < 0.01 which is significant

Discussion

The present study demonstrated an elevated mean concentration of inflammatory marker IL6 in the maternal plasma of preeclamptic patients when compared with healthy pregnant Women (Table-1, Figure-1). This increase was highly significant (P value < 0.0001). Our finding indicated that raised level of above marker during pre eclampsia, might be used as markers of inflammation and endothelial dysfunction in the preeclamptic pregnancies. This increase in concentration of IL6 suggested that abnormal cytokine responses in mother might be involved in the pathogenesis of pre eclampsia. Our finding was similar to previous studies done by many investigators. In 2014 by Arjun Jain, et al^[15], hypo perfusion induces a preeclampsia like inflammatory response. They found increase in the levels inflammatory cytokines and IL6 in pre eclampsia. In 2011 an study was done by Cristina et al^[16] on Inflammatory Disturbances in Preeclampsia: Relationship between Maternal and Umbilical Cord Blood. They found that level of pro-inflammatory cytokines IL-6, significantly higher in Pre- eclampsia pregnant women as compared with normotensive pregnant women. Sharma *et al.*^[17] demonstrated that IL-6 and TNF-a were higher in relation to normal pregnancy. These studies supported our study. But conflicting results were found regarding the role of IL-6 in pre eclampsia in different studies. In two different studies, Page et al^[18]. and Tosun^[19] et al. have shown that maternal serum levels of IL-6 significantly increased in pre-eclamptic patients rising in a way that

higher levels are found in patients with severe compared to mild preeclampsia. Studies done by Desai et al.^[20] and Maruo et al^[21] suggested that IL6 interferes with endothelial cell function and contributes to the systemic endothelial activation and vascular damage suggesting that preeclampsia is associated with elevated plasma IL6 levels. These findings are consistent with a role for pro inflammatory cytokines in the genesis of preeclampsia. It has been seen that IL6 can cause improper angiogenesis^[22], inhibit prostacyclin production by down regulating enzymatic activity of Cyclooxygenase and specifically enhances the endothelial cell permeability by altering the ultra structural distribution of tight junctions^[22]. But conflicting results were found regarding the role of IL-6 in preeclampsia in different studies [23,24].

Conclusion

We concluded from our study that probably increased lipid peroxidation of plasma membrane due to oxidative stress is an important factor in the pathogenesis of pre-eclampsia because lipid peroxides damage endothelial cells, produce vasoconstriction and inflammation. Increased IL6 level inhibits vascular remodeling and endothelial damage which may lead to pre-eclampsia. Thus possibly IL6 may be involved in the pathogenesis of pre-eclampsia.

As we have taken both study groups in their third trimester, to know the role of IL6 in early pregnancy and their implementation to control the incidence of pre eclampsia, is required further study.

Conflict of Interest: There is no conflict of interest in the study.

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