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A COMPARATIVE STUDY OF THE SERUM TNF-ALPHA AND SERUM INTERLEUKIN-10 LEVELS IN WOMEN WITH PRE-ECLAMPSIA AND NORMAL HEALTHY PREGNANT WOMEN

Biochemistry

G. G. Kaushik Department of Biochemistry, J.L.N. Medical College, Ajmer, Rajasthan, India

Madhu Sharma* Department of Biochemistry, J.L.N. Medical College, Ajmer, Rajasthan, India *Corresponding Author

Ankur Mathur Department of Biochemistry, Dr. S.N. Medical College, Jodhpur, Rajasthan, India

ABSTRACT

Aim: To assess variation in circulatory concentrations of cytokines such as Tumour necrosis factor α (TNF-α) and interleukin -10 (IL-10) in women with pre-eclampsia.

Methods: A hospital based observational study was conducted on 140 patients of pre-eclampsia and 140 normal healthy pregnant women. Serum AST, serum ALT, serum uric acid and urine protein were estimated by spectrophotometry and TNF-α and interleukin -10 were estimated by enzyme linked immunosorbent assay (ELISA).

Results: The mean serum levels of TNF- alpha and Interleukin -10 in pre-eclamptic women were 41.02 ± 11.33 pg/ml and 2.1 ± 0.72 pg/ml while in normal healthy pregnant women these were 15.37 ± 8.42 pg/ml and 4.32 ± 0.98 pg/ml respectively. These values were found to be statistically highly significant. ($p<0.0001$).

Conclusion: In this study, the level of TNF –α was increased whereas level of interleukin -10 was decreased in women with pre- eclampsia in comparison with normal healthy pregnant women.

KEYWORDS

Pre-eclampsia, TNF- α, Interleukin -10

INTRODUCTION

Pre-eclampsia (PE) is a pregnancy-specific syndrome, characterized by hypertension and proteinuria after the 20th week of gestation. This disease affects 3–14% of pregnancies worldwide, and there is a 7.5 to 65% risk of recurrence. It is one of the main causes of maternal and perinatal morbidity and mortality [1].

Clinically, mild pre-eclampsia is the first onset of this disease and is associated with hypertension ≥140/90mmHg and proteinuria ≥0.3 < 5g/24h occurring after the 20th gestational week in women previously identified as normotensive with no protein in urine. The associated symptoms are generalized edema, headache and blurred vision, and in severe cases, it may cause liver failure, kidney disease, coagulation disorders, respiratory distress syndrome and intrauterine foetal growth restriction [2]. Despite many hypotheses, the pathogenesis of pre-eclampsia has not been clearly established, and the most effective 'remedy' is delivery [3].

Tumour necrosis factor-α (TNF-α) is an inflammatory cytokine produced by human uterine and placental cells at the early and late stages of gestation and promotes the regulation of trophoblast growth and invasion. Furthermore, TNF-α which is a Th1 type cytokine induce trophoblastic apoptosis, inhibit differentiation and invasion of trophoblast and finally, may be involved in the incomplete invasion of trophoblast to spiral arteries; pathologic processes which leads to PE [4, 5, 6]. Pre-eclampsia is an endothelial disorder and TNF plays a significant role in changing the balance between oxidant and antioxidant, the pattern of prostaglandin production and expression of adhesion molecules in blood vessels [7,8].

Interleukin-10 (IL-10) as an anti-inflammatory cytokine is produced by some subpopulations of T lymphocytes, monocytes, macrophages, and cytotrophoblasts [9]. As IL-10 has strong suppressor activity on pro-inflammatory cytokines TNF-α and interferon-gamma (IFN-γ), it is suggested that placental hypoxia leads to insufficient IL-10 production, resulting in increased or uncontrolled production of pro-inflammatory cytokines [10].

The quantitative importance of TNF-α, IL-10 and biochemical factors that mediate vascular dysfunction and hypertension during pre-eclampsia remains to be elucidated. This study was undertaken to evaluate the serum levels of TNF-α, IL-10 and other inflammatory markers to assess if infection – inflammation is a major risk factor in development of pre-eclampsia.

MATERIAL AND METHODS

The present study was conducted on pre-eclamptic pregnant women attending or admitted in OPD or wards of Government Mahila Chikitsalya, Ajmer. Total 140 Cases of pregnancy induced hypertension (PIH) chosen were primiparous and as per standard description. Age and gender matched 140 controls were included in the study.

Normal pregnancy was diagnosed on the basis of clinical, biochemical and ultrasonographical findings. Patients of Primigravida with an arterial blood pressure of at least 140 mm Hg systolic and 90 mm Hg diastolic on two occasions 24 hours apart after 24 weeks of gestation with a total protein excretion of greater than 300 mg/day and classical edema or as excessive weight gain around third trimester of pregnancy were included in this study.

Patients with a history of Pre-existing diabetes, Gestational diabetes, Thyroid disorders, Renal disease, multiple pregnancies, chronic hypertension, Connective tissue diseases, Viral diseases, Urinary tract infection, Toxoplasmosis, Thrombocytopenia, Coagulation disorders, Other cardiovascular illness and symptomatic infectious Pregnant patients diagnosed with chromosomal aberrations before or after childbirth and BMI>30 were excluded.

A written informed consent was taken from all patients fulfilling the inclusion criteria, followed by detailed history and examination.

Fasting blood samples were collected from subjects by venepuncture in plain vials at the last trimester of pregnancy period. None of the participants were in labor at the time of sample collection. Serum was separated after one hour; early morning midstream urine sample in a sterile container without preservative was collected for the estimation of Urine protein. Estimation of Serum AST, serum ALT, serum uric acid and urine protein was done by spectrophotometry (Beckman coulter AU680) and TNF-α and interleukin -10 were estimated by enzyme linked immunosorbent assay (ELISA).

Statistical analysis

Data were recorded in a predesigned performa and managed in an excel spread sheet. Data were reported as mean ± SD. Comparison of physical and biochemical parameters between normal healthy pregnant women and pre-eclamptic subjects was performed using equal or unequal variance unpaired student t-test for continuous variables (as applicable).

All p-values were based on a two sided test of statistical significance. Significance was accepted at the level of $p<0.05$.

RESULTS AND DISCUSSION

The present study has been conducted to evaluate TNF–α and IL-10 in pre-eclamptic patients. Pre-eclampsia is pregnancy specific syndrome

characterized by new onset hypertension and proteinuria occurring usually after twenty weeks of gestation. Its early detection and timely management is the most important step to overcome this obstacle.
This study consisted of 140 cases of normal healthy pregnant women and 140 cases of pre-eclampsia.

Table No. 1 Anthropometric parameters in normal healthy pregnant women and pre-eclamptic subjects

Parameters	GROUP I Normal Healthy pregnant women Mean ± SD (n=140)	GROUP II Pre-eclamptic subjects Mean ± SD (n=140)	'P' value*
Age (Years)	28.03 ± 3.92	27.85 ± 4.73	0.72
BMI (Kg/m2)	23.35 ± 3.04	27.78 ± 4.12	<0.0001
Systolic B.P. (mmHg)	118.52 ± 4.03	153.88 ± 12.56	<0.0001
Diastolic B.P.(mmHg)	71.74 ± 6.32	100.69 ± 13.01	<0.0001

*p- value < 0.0001 Highly significant (HS)
p- value < 0.01 Significant (S)
p – value > 0.05 Non significant (NS)

The anthropometric parameters viz, age in years in group I was 28.03 ± 3.92 and in group II was 27.85 ± 4.73 in our study, BMI in kg/m^2 in group I was 23.35 ± 3.04 and in group II was 27.78 ± 4.12, which was significantly higher in pre-eclamptic subjects (p <0.0001).

Systolic blood pressure of pre-eclamptic patients in mmHg (153.88 ± 12.56) was higher and statistically significant (p < 0.0001) than normal healthy pregnant controls (118.52 ± 4.03). Diastolic blood pressure of pre-eclamptic subjects in mmHg (100.69 ± 13.01) was also higher and statistically significant (p < 0.0001) than normal healthy pregnant controls (71.74 ± 6.32).

Our findings are in line with the Pinheiro Melina B et al. (2013) who observed significantly higher systolic and diastolic blood pressures in pre-eclamptic subjects in comparison to normal healthy pregnant controls [11].

Table No. 2 Levels Of Various Parameters In Normal Healthy Pregnant Women And Pre-eclamptic Subjects

Parameters	GROUP I Normal Healthy pregnant women Mean ± SD (n=140)	GROUP II Pre-eclamptic subjects Mean ± SD (n=140)	'P' value*
Aspartate aminotransferase (U/L)	29.45 ± 4.46	59.01 ± 13.56	<0.0001
Alanine aminotransferase (U/L)	20.23 ± 2.72	49.12 ± 17.17	<0.0001
Uric acid (mg/dl)	3.7 ± 1.9	7.6 ± 1.2	<0.0001
Urine protein (gm/24hr)	0.04 ± 0.01	2.2 ± 1.3	<0.0001
TNF-α (pg/ml)	15.37 ± 8.42	41.02 ± 11.33	<0.0001
IL-10 (pg/ml)	4.32 ± 0.98	2.1 ± 0.72	<0.0001

*p- value < 0.0001 Highly significant (HS)
p- value < 0.01 Significant (S)
p – value > 0.05 Non significant (NS)

Comparison of various biochemical parameters in normal healthy pregnant controls and pre-eclamptic subjects is shown in table no. 2.

The mean serum aspartate aminotransferase levels of normal healthy pregnant controls and pre-eclamptic subjects were 29.45 ± 4.46 and 59.01 ± 13.56 respectively. The mean serum Aspartate aminotransferase level was higher in pre-eclamptic subjects and statistically significant (p <0.0001). A similar trend was observed in mean serum alanine aminotransferase (U/L) level in group II (49.12 ± 17.17) when statistically compared with group I (20.23 ± 2.72). Munazza B et al. (2011) in their study reported that mean values of enzyme ALT in cases was 55.81 ± 31.93 U/L while in the controls it was 15.22 ± 3.30 U/L (p < 0.001). Mean serum AST in the cases was 41.34 ± 10.76 U/L and in the controls it was 24 ± 2.54 U/L (p < 0.001), which is similar to our findings [12].

Table no. 2 shows statistically significant and higher mean serum uric acid levels (mg/dl) in group II (7.6 ± 1.2) when compared with group I (3.7 ± 1.9) ;(p < 0.0001). A study on 215 hypertensive pregnant women in their 3rd trimester showed hyperuricemia and renal involvement in pre-eclampsia [13]. Mustaphi et al. (1996) showed uric acid level as one of the parameter used in early diagnosis of pregnancy induced hypertension [14].

Our findings show statistically significant and higher mean urine protein (gm/24hr) in group II (2.2 ± 1.3) when compared with group I (0.04 ± 0.01) ;(p < 0.0001). Mean urine protein value was higher in pre-eclamptic pregnant subjects than normal healthy pregnant subjects that showed proteinuria condition which is a characteristic feature of pre- eclampsia.

The pathophysiologic events responsible for the increase in urinary protein excretion in pre-eclampsia are complex and not entirely understood. Possible increases in glomerular capillary pressure, alterations in the size and/or charge selectivity of the glomerular filter, compromised proximal tubular reabsorption are the most often posited mechanisms. Although the possible change in charge selectivity of the glomerular barrier in pre-eclampsia is still a subject of active debate, studies have shown that despite 100-fold increases in urinary albumin excretion, fractional excretion of neutral dextrans of comparable molecular weight was decreased [15]. This loss of charge selectivity in pre-eclampsia could thus explain why the relative excretion of neutral dextrans decreases while excretion of total proteins increases.

The mean serum TNF-α (pg/ml) levels of normal healthy pregnant controls and pre-eclamptic patients were 15.37 ± 8.42 and 41.02 ± 11.33 respectively. The mean TNF-α level was higher in pre-eclamptic patients and statistically significant (p <0.0001).

Many authors suggest that elevated circulating TNF-alpha levels may be involved in the pathogenesis of pre-eclampsia [16, 17, 18]. These results are in line with previous studies that found higher concentrations of TNF-α, circulating chemokines and adhesion molecules in women with pre-eclampsia, compared to controls with normal pregnancy.

Several studies have shown an association between pre-eclampsia and high plasma and serum concentrations of TNF-α [19, 20, 21, 22]. TNF- α has cytotoxic action and appears to inhibit trophoblastic invasion [23] and promote endothelial activation [24], contributing to an altered production of prostaglandin and an imbalance between vascular regulatory factors [25].

The mean serum IL-10 (pg/ml) levels of normal healthy pregnant controls and pre-eclamptic subjects were 4.32 ± 0.98 and 2.1 ± 0.72 respectively. The mean level of IL-10 was lower in pre-eclamptic patients and statistically significant (p <0.0001).

Kalantar et al. 2013 found that higher levels of IL-10, which is an anti-inflammatory cytokine in sera of normal pregnant women [26]. In concordance with our results, Sharma et al. (2007) showed that IL-10 was decreased significantly in pre-eclampsia patients compared to normal pregnant women [21]. These findings emphasize on the protective role of IL-10 in pre-eclampsia [27].

Inversely, Bakheit et al. (2009) [28] and Xie et al. (2011) [29] found higher serum levels of IL-10 in pre-eclamptic women and Jonsson et al. (2006) observed no significant difference in IL-10 levels between normotensive and pre-eclamptic women [30].

These contradictory results of various studies could be related to sampling in different stages of pregnancy.

The overall findings of the present study, thus confirm that serum TNF –α is significantly higher in pre –eclamptic patients. But the serum levels of IL-10 are significantly lower in the pre-eclamptic subjects. The study therefore recommends research on larger patient group to evaluate the association of pro and anti-inflammatory markers in pre-eclamptic patients.

In pre-eclampsia, the inflammatory process alters the balance between Th1 and Th2 cytokines, resulting in the predominance of Th1. Thus, the cascade of production of pro-inflammatory cytokines involved in the disease is initiated or intensified.

In normal pregnancy, the trophoblasts invade the endovascular tissue and replace in the uterine arterioles. It seems that in pre-eclampsia T helper 1 – type cytokines induce trophoblastic apoptosis, inhibit

differentiation and invasion of trophoblast, and finally, may be involved in the incomplete invasion of trophoblast to spiral arteries. It may cause placental hypoxia which leads to insufficient IL-10 production. IL-10 has strong suppressor activity on pro-inflammatory cytokine TNF-α. Insufficient IL-10 production results in increased or uncontrolled production of TNF-α aggravating the disease.

CONCLUSION

Pre-eclampsia is a multisystem disorder based on the cascade immunological events originating from the ischemic placenta. The identification of inflammatory markers (TNF- α and IL-10) will contribute to a better understanding of the pathophysiology of pre-eclampsia and will give us a clinically validated screening procedure for a better management of this disorder. In addition the early identification of high-risk cases will offer the opportunity for prophylactic therapy, thus improving the perinatal outcome.

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