SERUM LEPTIN LEVELS AS A MARKER FOR SEVERITY OF PRE-ECLAMPSIA

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ABSTRACT

Introduction: Pre-eclampsia is one of the most common clinical conditions seen in high risk pregnancies with 7-10% complication rate. Preeclampsia is characterized by hypertension and arteriolar vasoconstriction which decreases the uteroplacental perfusion and results in placental hypoxia. Long lasting placental hypoxia can in turn lead to fetal growth retardation. Under the control of the Obesity gene, the adipose tissues and placental trophoblasts synthesize and secrete a protein called leptin. Leptin binds to receptors to initiate a cascade of biological processes involved in regulating food intake and energy expenditure. Leptin has been implicated in the pathogenesis of pre-eclampsia.

Objective: To determine the role of serum leptin levels as a marker for severity of Preeclampsia.

Material and Methods: This study was conducted at Gynae OPD Hayatabad Medical Complex and Lady Reading hospital Peshawar for a period of 6 months. This was a case control study in which the sample size was 80 subjects. These were divided into two groups, 40 cases and 40 controls. Inclusion criteria include all patients with mild and severe pre-eclampsia having gestational age more than 20 weeks.

Exclusion criteria were twin pregnancies, patients having heart disease or kidney disease and gestational age of less than 20 weeks. Leptin level in serum was estimated by ELISA, and proteinuria by dipstick method.

Results: Total of 80 subjects (40 cases and 40 controls) participated in this trial as per inclusion and exclusion criteria. Cases were divided into mild & severe pre-eclampsia. The mean age of cases was 27 years and controls were 24.9 years. Mean period of gestation of both cases and controls was 35.3 weeks. Mean B.P of cases was 153/100 mmHg, while that of controls was 111/75mmHg. The mean leptin levels were 9.57 ng/ml in controls. In mild pre-eclampsia, leptin levels were 25.57 ng/ml and in severe pre-eclampsia, the levels were 36.47 ng/ml. Serum levels of leptin were found to be significantly associated with severity of pre-eclampsia. Risk estimation showed that each 15 ng/ml increase in severe leptin levels will increase the risk of pre-eclampsia by four fold (OR=4.9) Reference range of leptin levels in normal pregnancy was found to be 8-11 ng/ml. In mild pre-eclampsia it was found to be 21-30 ng/ml while in severe pre-eclampsia, the range was 33-40 ng/ml.

Conclusion: From this comparative study it is concluded that serum leptin levels may be used as a marker for severity of pre-eclampsia.

INTRODUCTION

Pre-eclampsia is one of the most common clinical conditions, seen in high risk pregnancies[1] with 7-10% complication rate. Pre-eclampsia, is characterized by development of hypertension and proteinuria which is noted after 20 weeks of gestation in a previously normotensive, non proteinuric pregnant women. According to international society for the study of hypertension it is defined as blood pressure ≥140/90 mmHg on 2 separate occasions 6 hours apart or a single recording of a diastolic blood pressure of 100 mmHg in association with proteinuria >1 + on dipstick testing[2].

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The etiology of the pre-eclampsia remains unclear. Several genetic, placental, metabolic and immune factors are postulated to give rise to abnormal remodeling of the placental vasculature[1]. Pre-eclampsia is considered to be consisting of a two stage disease. Stage 1 is characterized by a reduction in placental perfusion. Stage 2 is the clinical syndrome[3]. The discovery of new serum markers might be helpful in identifying subjects at increased risk of developing pre-eclampsia.

Leptin is a product of ob (obese) gene and a hormone that is synthesized mostly in adipose cells, mainly by white adipose tissues[4]. Leptin is also produced by human placental trophoblasts[5]. It may affect a number of processes in this organ, including angiogenesis, growth and immunomodulation. It has been suggested that leptin may be involved in the pathogenesis of pre-eclampsia by lipid peroxidation in cultured human

endothelial cells, and consequently promoting athero-genesis[6].

Studies have shown the high maternal leptin concentrations in the second trimester of pregnancy in women with pre eclampsia.[7] Further more,data that leptin levels are associated with increased Plasmino-gen Activator inhibitor 1 (PAI) levels in non-pregnant individuals,suggest a potential relation between this marker and the coagulation disturbance characteristic of pre-eclampsia.[8]

One of the studies has shown that plasma leptin level in early gestational age precedes the significant risk of pre eclampsia and can be used as a marker of pre eclampsia[9], while another report that each 10ng per ml rise in leptin concentration was associated with 30% increase in pre eclampsia risk.[10] Plasma leptin level can also be taken as a marker for severity of pre-eclampsia and intra- uterine growth restriction[11]. Few studies, mainly cross-sectional in their design have evaluated changes in serum leptin in pre-eclamptic women and have given rise to conflicting results[12,13,14].

Serum leptin levels determination in Pakistani pregnant females was also done and it suggests that leptin does play a role in body weight and energy reg-ulation during pregnancy. The significant positive cor-relation between leptin and placental weight suggests that placenta may be the site of synthesis and secretion of leptin during pregnancy.[13] The purpose of this study is to establish the possible role of serum levels of leptin as a marker of pre-eclampsia.

RESEARCH METHODOLOGY

MATERIALS AND METHODS

Subjects:

A total of 40 pregnant female with pre-eclampsia were selected for this case control study from the ad-mitted patients and patients visiting out patient depart-ments of tertiary care hospitals of Gynae and obstetrics unit Hayatabad Medical Complex and Lady Reading Hospital as per the inclusion and exclusion criteria. These patients were compared with 40 pregnant female without pre-eclampsia. After the full explanation of the study, written informed consent was obtained from each study subject.

SAMPLE SELECTION:

CASES:

Inclusion Criteria:

* All pre-eclampsia patients from 20-35 years age. Cases will be identified and grouped based on maternal criteria for pre-eclampsia.

1) Mild pre-eclampsia was defined as elevated blood pressure (140/90mmHg or greater on two mea-surements that are obtained six or more hours apart, with proteinuria equal +1 on urine dipstick testing.

2) Severe pre-eclampsia is defined as blood pres-sure ≥160/100 mmHg with greater than +1 urine dipstick testing or ≥140/90mmHg on two occa-sions 6 hours apart and anyone of the following: platelets < 120,000/ml, aspartate aminotrans-ferase (AST) >45 U/L, alanine aminotransferase (ALT) > 60 U/L, and/orcreatinine ≥1mg/dl.

* Gestational age more than 20 weeks.

Exclusion Criteria:

* Twins/triplets pregnancy.

* Having heart disease, kidney diseases and liver diseases with essential HTN.

CONTROLS:

Inclusion Criteria:

All normotensive pregnant females with gesta-tional age more than 20 weeks.

Exclusion Criteria:

• Twins/triplets pregnancy.

• Having heart disease, kidney diseases and liver diseases with essential HTN.

• Diabetic patients.

BIOCHEMICAL ANALYSIS:

Serum leptin levels were measured by enzyme linked immunosorbent assay (ELISA), which is an en-zyme immunoassay for the quantitative measurement of serum/plasma leptin.

The kit used was leptin ELISA kit (IBL Internation-al, Germany RE53151), which is a solid phase enzyme linked

immunosorbent assay (ELISA) based on the sandwich principle.

Urine proteins were determined through dipstick method.

ETHICAL CONSIDERATION: The present study was approved by institutional ethical committee of Postgraduate Medical Institute.

STATISTICAL ANALYSIS: Data was entered and analyzed into statistical product for services solutions (SPSS) version 17. All the variables were categorized in appropriate categories.

Serum Leptin levels was a continuous variable. It was estimated in two groups, Cases and Controls. Cases were further divided into two categories i-e Mild pre-eclampsia and Severe pre-eclampsia.

Serum leptin levels were estimated in all three

groups of patients. Probability value (p value) of less than 0.05 was taken as statistically significant. For descriptive analysis Means and standard deviation were calculated for numerical variables and proportion of all the categorical variables in the study.

P-values were determined using chi-square test for categorical variables, while T-test was applied for determination of continuous variable. The risk estimation was done by comparing cases and controls through odds ratio.

RESULTS

- Mean age of cases was 27 ± 5.3 years while mean age of controls was 24 ± 5.2 years.

- Mean age at marriage of cases was 20 ± 3.4 years, while mean age of controls was 21.8 ± 3.7 years.

- Mean period of Gestation of cases was 35.3 ± 2.8 weeks, while mean period of gestation of controls was 35.3 ± 2.9 weeks.

- Mean weight of cases was 74 ± 16.8Kg, while mean weight of controls was 72 ± 9.8 Kg.

- Mean leptin levels in cases was 32.3 ± 14.6 ng/ml, while in controls was 9.5 ± 8.9 ng/ml.

The cases were categorized in mild and severe pre-eclampsia and association of both the groups with leptin levels were found. Leptin levels obtained were divided into 3 categories. 1st group contained subjects with levels < 15ng/ml, 2nd group contained subjects with levels ranging from 15-35ng/ml, and the 3rd group with levels >35ng/ml. The results showed more subjects 47.5% in 3rd group, 37.5% in 2nd group, while 15% in 1st group.

The risk of pre-eclampsia increases 4-5 fold, with each 15ng/ml increase in serum leptin levels.

Table 1: SOCIO DEMOGRAPHIC AND REPRODUCTIVE CHARACTERISTICS OF THE STUDY SUBJECTS

	Group	No of patients	Mean	Standard Deviation(SD)
Age (years)	Cases	40	27.02	5.33
	Control	40	24.90	5.23
Age at Marriage (years)	Cases	40	20.07	3.45
	Control	40	21.85	3.79
Period of Gestation (weeks)	Cases	40	35.35	2.86
	Control	40	35.30	2.93
Weight (kg)	Cases	40	74.67	16.89
	Control	40	72.35	9.82
Leptin level (ng/ml)	Cases	40	32.38	14.69
	Control	40	9.57	8.95

Table2: DISTRIBUTION OF LEPTIN LEVELS IN MILD AND SEVERE PRE-ECLAMPSIA

	Leptin level		
	<15 ng/ml	15 – 35 ng/ml	>35 ng/ml
Mild	3 (20%)	9 (60%)	3 (20%)
Severe	3 (12%)	6 (24%)	16 (64%)
Total	40 (100%)		

Table 3: RISK ESTIMATION OF PREECLAMPSIA WITH SERUM LEPTIN LEVELS

Risk Estimate	
	Value
OR (<15.00 / 15.01+)	4.9
N of Valid Cases	80

DISCUSSION

Pre-eclampsia is a complication in pregnancy caused by factors released from a dysfunctional placenta[15]. Delivery of the placenta results in clinical resolution, so placenta is viewed as the essential organ in the development of pre-eclampsia[16].

Leptin is described as a major placental protein which exhibits metabolic and physiologic functions in a normal pregnancy. However, clinical and experimental trials have not yet clarified the definite role of leptin in the pathophysiologic mechanisms of high risks pregnancies[17,18]. There is a possibility that leptin like several others placentally derived substances (e.g. steroids, hormones and cytokines) may be involved in the pathogenesis of pre-eclampsia. In a longitudinal analysis of maternal plasma leptin concentrations during normal pregnancy and pre-eclampsia done by Anim Nyame in 2000 determined serum leptin levels in 19 women with

pre-eclampsia and 13 normal pregnant controls found that leptin concentrations were significantly greater in women with pre-eclampsia than in pregnant women without pre-eclampsia (with a p-value of <0.001). This study reports for the first time that leptin concentrations are elevated before pre-eclampsia is clinically evident09. In another study by Highman et al in 1998 have failed to observe such a relationship between leptin concentrations in pre-eclampsia and maternal BMI[19].

Ouyang Y et al in their study also found plasma leptin concentrations to be significantly higher in women diagnosed with mild and severe pre-eclampsia (with a p-value of p<0.05 and p<0.01) respectively[14]. A study by Iftikhar U et al was conducted in which total 90 patients were analyzed. Forty five were normotensive and forty five were pre-eclamptic patients. Twenty eight were mild pre-eclampsia and seventeen were having severe pre-eclampsia. Maternal serum leptin levels were significantly higher in pre-eclamptic group 62.1 ± 23.8 than in control group 26.8 ± 6.47. According to the severity of pre-eclampsia, the serum leptin levels were found to be statistically higher in severe group 85.7 ± 13.3 than in mild group 47.7 ± 15.9. Proteinuria of 3± was found to be significantly associated with serum leptin levels[16].

Under these circumstances the current study was undertaken to compare and confirm the reported increase in serum leptin levels both in pre-eclampsia and normal pregnancies. The results of this study shows that levels of serum leptin were elevated when compared to normotensive pregnancies (p-value <0.001). It was also found that there was a strong linear component of trend in risk of pre-eclampsia with maternal rise of plasma leptin concentration. (Odds ratio 4.9 when serum leptin levels increase by 15ng/ml), moreover, the leptin levels were compared between mild and severe pre-eclampsia and highly significant difference was found between the two groups. The results of our study were in concordance with the studies[1,9,10,14,78] having increase in leptin concentrations during pre eclampsia.

CONCLUSION

From this comparative study it is concluded that the increased levels of leptin in severe pre-eclampsia as compared to mild pre-eclampsia can be helpful in the use of serum leptin levels as a maker for severity of pre-eclampsia.

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