Serum Leptin Levels In Normal Pregnancy and In Patients with Preeclampsia – A Comparative Study

Dr.B.Aradhana [MD][1], Dr.V.Ratnakumari [MD][2],Dr.N.Vani [MD][3]

1 Assistant professor,Department of Biochemistry,Government medical college,Nalgonda,India.
2 Associate professor , Department of Biochemistry,Osmania medical college,Hyderabad,India.
3 Professor, Department of Biochemistry ,Hyderabad
Corresponding Author: Dr. V.Ratnakumari [MD]

Abstract:

Background: *Pre-eclampsia remains the leading cause of maternal and perinatal mortality and morbidity which complicates 2-8% of all pregnancies. Leptin is described as a major placental protein which exhibits metabolic and physiological functions in a normal pregnancy. In preeclampsia, placental production of leptin is increased under hypoxic conditions, so it is used as a marker of placental ischemia.*

Objectives: *The objective of the present study is to compare the serum leptin levels in normal pregnancy and in patients with preeclampsia and define the role of leptin as a marker for prediction of preeclampsia. Serum uric acid and proteinuria were also estimated.*

Methods: *A case control study was done with 87 patients divided into 2 groups. 43 normotensive pregnant women served as controls and 44 preeclamptic women served as cases. Serum Leptin was estimated by ELISA method. Serum Uric acid was estimated by uricase method and 24 hour Urinary proteins were estimated by using 3% SSA method .Data was analysed by using graph pad prism version 7.0.*

Results: *In the present study, women with Preeclampsia had significantly higher levels of serum Leptin (70.63 ± 19.65), serum Uric acid (6.41± 0.71) and 24 hour urinary proteins (505.3 ± 118.8) when compared to normotensive pregnant women serum leptin (38.78 ± 17.93), Serum Uric acid (3.8± 0.52) and 24 hour Urinary proteins (19.52 ± 4.36) with the p value < 0.0001.*

Interpretations & Conclusion: *Women with preeclampsia had significantly elevated levels of serum leptin when compared to normotensive pregnant woman suggesting the role of ischemia/hypoxia in upregulating the placental production of serum leptin. Thus, serum leptin may be used as a marker for prediction of preeclampsia and may be its severity. Early diagnosis of preeclampsia and thus early management will reduce the maternal and perinatal morbidity and mortality.*

Key words: *Preeclampsia, serum leptin, hyperuricemia.*

I. Introduction

Preeclampsia is best described as a pregnancy-specific syndrome that develops after 20 weeks of gestation, affects both the mother and the unborn baby and virtually every organ system. [1] According to International Society for the study of Hypertension, Preeclampsia is defined as blood pressure $\geq 140 / 90$ mmHg on 2 separate occasions 4hrs apart or a single recording of a diastolic blood pressure of 110mmHg, in association with proteinuria $\geq 2+$ ondipstick testing [2]in a previously normotensive patient.

Preeclampsia affects 8–10 % of all pregnancies worldwide, contributing substantially to the prenatal morbidity and mortality of both mother and newborn.[1] More than half of these hypertensive deaths were preventable and therefore it places great demands for maternity care because the onset and clinical course are unpredictable, and there is currently no predictive test available.[3]

The pathophysiology of preeclampsia includes an early placental dysfunction, characterized by insufficient invasion of the spiral arteries by the trophoblast, placental ischemia, and impaired perfusion of the uteroplacental unit which can lead to fetal growth restriction.[1]

Leptin, a novel Hormone is a 16Kda non-glycosylated polypeptide product of obese (Lep[ob]) gene, belonging to helical cytokine superfamily is mainly but not exclusively produced and secreted by adipocytes in proportion to fat mass.[2,4,5] It is expressed abundantly and specifically in the adipose tissue [4].The lep[ob] gene encodes 167 amino acids which is located on the long arm of chromosome number 7,[2,6] and plays an important role in modulating satiety, energy homeostasis, and reproductive biology.[7]

Leptin is described as a major placental protein which exhibits metabolic and physiological functions in a normal pregnancy. The proposed roles for leptin in pregnancy include the regulation of fetal growth,

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placental angiogenesis, growth and immunomodulation, as well as mobilization of maternal fat. Thus, placenta is a target of leptin action as well as a source for leptin synthesis. [3]

In PE, the maternal plasma leptin concentrations are elevated when compared to normal pregnant women .[8] This increase is possibly because of augmented placental production of hormones under hypoxic condition., which is a consequence of reduced placental perfusion.[2]It is plausible that elevated leptin levels in maternal circulation may aggravate hypertension, as leptin activates the sympathetic nervous system and stimulate catecholamine secretion and so it may be used as a marker of placental ischemia.[2]

There are several potential origins for uric acid in preeclampsia; abnormal renal function, increased tissue breakdown, acidosis and increased activity of the enzyme xanthine oxidase/dehydrogenase.[9,10,11] Serum uric acid levels have been also known to increase with increasing severity of the disease.[12] The increased frequency of preterm birth and growth restriction was present in hypertensive women with elevated concentration of uric acid even in the absence of proteinuria in women who go on to develop preeclampsia.[9]

In normal pregnancy, urinary protein excretion increases substantially; hence, total protein excretion is considered abnormal in pregnant women when it exceeds 300 mg/24 hours.[13]

Hence the present study is designed to evaluate the relationship between serum leptin levels in normal pregnancy and in patients with preeclampsia.This study has predictive as well as Therapeutic needso as to develop new strategies for prevention of complications and early initiation of treatment. Serum uric acid and proteinuria were also compared.

II. Materials And Methods

2.1 Setting: A case control study was conducted in the Department of Biochemistry, Osmania General Hospital, Hyderabad after Ethical clearance was obtained for the study from the institutional ethical committee.

2.2 Sources of samples and Data:Samples were collected from the antenatal women attending modern maternity hospital,Petlaburj.Informed consent was taken from all individuals who took part in the study. Samples were analysed at Department of Biochemistry, Osmania General Hospital .

2.3 Grouping:A study of 87 subjects divided into 2 groups with 44 cases and 43 controls.
Group 1 : 43 normotensive pregnant women served as controls.
Group 2 : 44 pregnant women with preeclampsia served as cases.

2.4 Inclusion criteria :1.Normotensive pregnant woman > 20 weeks of gestation.
2.Preeclamptic pregnancy (of more than 20 weeks gestation)
3. Age group : 18 – 35 years

2.5 Exclusion criteria:1. Twin pregnancy
2. Gestational Diabetes
3. Chronic hypertension
4. Liver failure
5.Renal failure

2.6 Procedure methodology :
2.6.1) Blood sample: 5 ml of venous blood was collected under aseptic precautions into non vacuum plain serum tubes. Collected blood was allowed to clot and then centrifuged and serum was separated. Serum was analysed immediately for uricacid and the remaining serum was stored in an aliquot at -20°C for analysing serum Leptin concentration.

2.6.2) Urine Sample: 24 hour urine sample was collected in a sterile container with added preservatives after giving proper instructions to the patient.
Serum leptin was analysed by Enzyme linked immunosorbent assay(ELISA).[1] Serum uric acid was analysed by Uricase/Peroxidase Method or Modified Trinder Method.[14,15]Urinary protein concentration were analysed by3% Sulpho Salicylic Acid (SSA) Method .[14,16]

2.7 Statistical analysis: The data was analyzed using Graph Pad Prism software version 7.0. The results were expressed as Mean and Standard deviation of various parameters in different groups.

III. Results

Table1: Study parameters in all the groups

Parameters	Group 1			Group 2		
	MEAN	S.D	SEM	MEAN	S.D	SEM
1.SerumLeptin (ng/ml)	38.78	17.93	2.735	70.63	19.65	2.963
2.Serum uric acid (mg/dl)	3.809	0.5255	0.8013	6.416	0.7104	0.1071
3. 24 Hour Urinary Proteins(mg/day)	19.52	4.368	0.6662	505.3	118.8	17.91

The Mean ± SD of all the parameters studied in total cases were high when compared to those of controls.

In order to assess the significance of the differences observed in the mean values of different parameters observed in different groups studied, the data was subjected to unpaired t test. The significance of difference of mean values of different groups and within the groups was represented by p values and p value <0.05 was considered as significant.

Table 2 : Unpaired t test values between group 1 and group 2

Parameter	t Value	P Value	Degrees of freedom (DF)
Serum Leptin	7.9	<0.0001(****)	84.61
Serum Uric Acid	19.49	<0.0001(****)	79.21
24hr Urinary Protein	27.11	<0.0001(****)	43.12

(**** - Significant)

Mean $\pm$ SD of serum leptin ,serum uricacid and 24hour urinary proteins levels are significantly elevated in preeclamptic group when compared to normotensive pregnancy group.

Table 3: Calculation of risk ratio of serum leptin in controls and cases

	ELEVATED LEPTIN	NORMAL LEPTIN	P VALUE
GROUP 2(Cases)	32	12	<0.0000001
GROUP 1(Controls)	6	37	<0.0000001

Risk ratio was calculated as 5.212 with the confidence intervals of 2.4 – 11.1 .From the risk ratio it can be said that elevation of serum leptin is 5 times more associated with preeclampsia than with normal pregnancy.
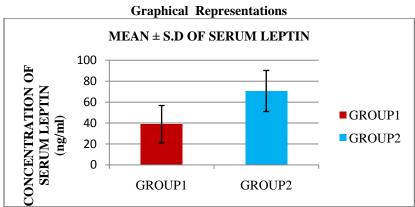
Graphical Representations

Fig 1: Graphical representation of mean $\pm$SD of serum leptin in 2 groups
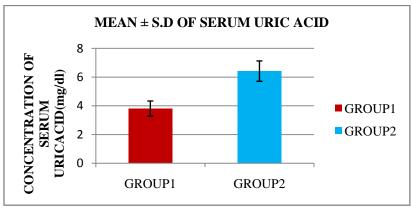

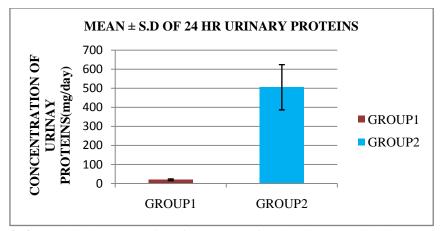**Fig 2 :**Graphical representation of mean $\pm$SD of serum uric acid in 2 groups

Fig 3 : Graphical representation of mean $\pm$SD of 24 hr urinary proteins in 2 groups

IV. Discussion

Pre-eclampsia is the most important cause of maternal morbidity and mortality globally. The cause of hypertensive disease is not yet clear; it includes immunological, genetic, environmental and placental abnormalities.[17]Diagnostic criteria for preeclampsia include new onset of elevated blood pressure and proteinuria after 20 weeks of gestation.[18] According to George and Granger in pre-eclampsia the placental tissues secrete some soluble secretions to the maternal blood. These secretions are the important pathological factors which cause high blood pressure by secretion of Endothelin -1(ET-1), a strong vasoconstrictor in blood.[17]

Leptin is an adipocyte-derived hormone that decreases food intake and body weight via its receptor in the hypothalamus. Its level increases in preeclampsia.[19]Higher concentration of leptin during early pregnancy is emerging as potential predictor for the development of PE.[20]

Impaired renal function is a pathophysiological component of preeclampsia and the increase in plasma leptin concentration may reflect reduced renal clearance.Also high leptin levels may be due to the possible haemoconcentration in preeclampsia caused by association of preeclampsia with reduced plasma volume.**[21]** Placental ischemia explains rapid increase in leptin concentration during late third trimester in preeclampsia. Placental hypoperfusion produces local hypoxia that consequently augments leptin gene expression in the placenta.[4,21]In a microarray analysis, LEP was upregulated approximately 44-fold. Enhanced expression of LEP mRNA transcripts in placental tissue from preeclamptic women, suggesting specificity of placental involvement.[22]In study by Anim- Nyame , it was found that plasma leptin concentrations were increased in established PE and reported for the first time that leptin concentrations were elevated before PE clinically evident.

The exacerbated increase of leptin in the maternal peripheral circulation, coupled with poor cytotrophoblastic invasion, typifies the preeclamptic state and serves as a marker for general placental insufficiency and poor placental perfusion.[22]

Mumtaz et al explained that over-expression of placental leptin and leptin receptors triggers nor-adrenaline turnover within the brown adipose tissue so that sympathetic activity is increased in the fetal maternal unit, stimulating fetal wastage and sudden intrauterine demise.[6]According to a study by Masuyama et al.,the elevated leptin levels may represent the adaptation mechanism of the foeto-placental system.Pre-eclampsia induces inflammatory mediators, such as tumor necrosis factor-α and interleukin-6, which may in turn trigger leptin release.[1]Papastefanou et al., described that if the serum leptin levels increases than normal in early pregnancy the risk for developing preeclampsia will be greater in third trimester of pregnancy.[23]

Uric acid is the end product of purine metabolism. [24]Several plausible sources for increased uric acid in women with preeclampsia include, the fetus, placenta, and maternal organs and vasculature.[9] Reduced uric acid clearance secondary to reduced glomerular filtration rate, increased reabsorption , decreased secretion , increased trophoblastic tissue shedding, endothelial dysfunction, and reduced blood flow in the fetomaternal unit have also been hypothesized as the underlying cause of hyperuricemia in this condition.[25]

Preeclampsia is a multisystem endothelial disease that leads to glomeruloendotheliosis, and in severe cases it may lead to renal impairment and failure. "Permeability" of the glomerular basement membrane to proteins, including albumin, is key to the diagnosis.[26] According to Sami Jan et al., One of the "cornerstones" of antenatal care includes a screening programme directed at the detection of pre-eclampsia with regular measurements of blood pressure and urinalysis for proteinuria.[27]

It is reported that leptin together with uric acid has a role in oxidative stress characterized by increased reactive oxygen compounds which are thought to have role in etiopathogenesis of preeclampsia. Leptin activates

the formation of reactive oxygen compounds via its functional receptors which are present on many cells . Leptin was proposed to be a risk factor for many complications such as hypertension and atherosclerosis through vascular inflammation that results from accumulation of reactive oxygen compounds in endothelial cells [12].

The present study was undertaken to study and compare the serum leptin levels in normal pregnancy and in patients with preeclampsia along with serum uric acid and 24hrs urinary proteinuria. Leptin and Uric acid levels which have been thought to have role in etiopathogenesis of preeclampsia were found significantly high in preeclamptic group in the present study. It may be determined that leptin levels increased together with increased serum uric acid levels.

V. Conclusion

In the present study, the mean $\pm$SD of serum leptin, serum uricacid and 24hr urinary proteins were significantly elevated in preeclamptic patients when compared with that of normal pregnancy. To conclude, serum leptin serves as a marker of placental ischemia and predictive marker of preeclampsia. According to few studies, serum leptin levels can be used as a prognostic marker and severity marker of preeclampsia which needs further prospective studies to confirm the findings. Hence its determination in high risk cases may prove beneficial for its early management as well as reducing its complications further reducing maternal and perinatal mortality and morbidity.

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