

EVALUATION OF SERUM LEPTIN LEVELS DURING NORMAL PREGNANCY AND IN PRE-ECLAMPSIA

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Background: The present study was designed to compare the serum leptin levels in normal pregnancies with those pregnancies which are complicated by pre-eclampsia and to investigate the possibility of leptin being a marker of severity of pre-eclampsia. **Methods:** This was a comparative cross-sectional study, conducted at the Department of Gynaecology and Obstetric, Jinnah Postgraduate Medical Centre (JPMC), Karachi. For this purpose 45 primigravidas with normal pregnancy and 45 primigravidas with pre-eclamptic pregnancy were selected who were in their third trimester. All the subjects were of the same maternal age, gestational age, height and weight. Serum leptin levels were determined by immunoassay. **Results:** The study included 90 patients, divided into two groups. The serum leptin levels were found to be elevated in pre-eclamptic group as compared to normal pregnancy. When comparison was made on the basis of severity of pre-eclampsia, the serum leptin levels were found to be increased in severe group as compared to mild group. **Conclusion:** From this study it was concluded that the levels of serum leptin were higher in pre-eclamptic group when compared to controls during the third trimester of pregnancy indicating the possible role of leptin in the pathogenesis of pre-eclampsia. Also the elevated level of leptin may help in the prediction of the disease and may serve as a marker of severity of pre-eclampsia.

Keywords: Leptin, pre-eclampsia

INTRODUCTION

Pre-eclampsia (PE), a syndrome of pregnant women, is one of the leading causes of maternal and foetal morbidity and mortality.¹ In PE, there is development of hypertension and proteinuria after 20 weeks of gestation in a previously normotensive, non-proteinuric pregnant woman.² According to International Society for the study of hypertension, it 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 $\geq 2+$ on dipstick testing.³ The clinical course of the disease is progressive and is characterized by continuous deterioration that is ultimately stopped only after delivery.⁴ Pre-eclampsia is characterized by widespread endothelial damage and dysfunction throughout the maternal circulation, resulting in classic manifestation of hypertension caused by vasoconstriction, proteinuria caused by glomerular damage and oedema caused by increased vascular permeability.³ Oedema is a non-specific sign of pre-eclampsia because it occurs in 35% of normotensive patients. Rapid weight gain of 5 pounds or more in 1 week is considered a warning sign.⁵ During normal pregnancy, production of prostacyclin and thromboxane A₂ (TxA₂) increases. Prostacyclin is a potent vasodilator and antiplatelet aggregate. TxA₂ is a vasoconstrictor and platelet aggregator. In PE, the ratio of TxA₂ to prostacyclin is increased which may increase the vascular tone and elevate the blood pressure.⁶ In PE endothelial dysfunction may be the common final pathway linking upstream metabolic perturbances to the clinical manifestations. The pathophysiological findings include reduced organ perfusion due to vasoconstriction

accompanied by reduced plasma volume, activation of coagulation cascade, increased responsiveness to circulating pressor agents like adrenaline, nor-adrenaline, Angiotensin-II.⁷

Leptin, a 16Kda non-glycosylated polypeptide product of obese (Ob) gene, is mainly produced and secreted by fat cells in proportion to fat mass.⁸ The gene encodes 167 amino acids which is located on the long arm of chromosome.^{7,9} It seems likely that placenta plays a role in increasing the maternal plasma leptin concentrations during pregnancy. The leptin concentration is increased in blood of women with PE compared with matched group of normal pregnant women.¹⁰ Cross-sectional studies suggest that leptin concentration peaks in the second trimester and remains elevated until parturition.¹¹

In PE, the maternal plasma leptin concentrations are increased possibly because of augmented placental production of hormones under hypoxic condition.¹² There is also evidence that inflammatory mediators increase plasma leptin concentration¹³ and cytokines produced by monocyte/macrophage (interleukin (IL)-1 α , IL- β , IL- δ and tumour necrosis factor (TNF- α) and by T-cells (IL-2, interferon (IFN) γ , IL-4, IL-6, IL-10) may be associated with PE.^{14,15} It has also been demonstrated that leptin increases in situations associated with higher levels of cytokines as in women with PE.¹⁶ It is plausible that elevated leptin levels in maternal circulation may aggravate hypertension, as leptin activates the sympathetic nervous system and stimulate catecholamine secretion.¹⁷

MATERIAL AND METHODS

This was a comparative cross-sectional study, conducted at the department of Gynaecology and Obstetric, JPMC. This study was performed on 90 pregnant women of age ranging between 16–32 years and gestational age between 28–38 weeks. Only primigravidas with singleton pregnancies were included.

Inclusion Criteria

- 45 normotensive primigravidas with singleton pregnancy in their third trimester.
- 45 pre-eclamptic primigravidas with singleton pregnancy in their third trimester.

Exclusion Criteria

It was strictly observed and includes the following

- Pre-existing chronic hypertension
- Pre-existing diabetes mellitus
- Gestational diabetes
- Any chronic renal disease

All the subjects were briefed about the nature of the study and an informed consent was taken from all the recruits.

The arterial blood pressure was measured using a simple mercury sphygmomanometer on right arm in a comfortable sitting position after 10 minutes of rest.

To perform dipstick urine analysis, multistix URS-10 test strips were used.

The blood samples were collected under strict aseptic measures. Each sample was labelled with patient’s name and identification number. Samples were analyzed in one run at the end of the study. Serum leptin was determined by immunoenzymometric assay. Data was analysed using SPSS 10.0.

RESULTS

In this study, 90 patients were analysed. Among which 45 were normotensive primigravidas and 45 were pre-eclamptic primigravidas. Out of 45 pre-eclamptics, 28 were with mild PE and 17 were having severe PE. Results are summarized in tables 1–4. Maternal serum leptin levels were significantly higher ($p<0.001$) in pre-eclamptic group 62.1 ± 23.8 than in control group 26.8 ± 6.47 (Table-1). According to the severity of PE, the serum leptin levels were found to be statistically higher ($p<0.001$) in severe group 85.7 ± 13.3 than in mild group 47.7 ± 15.9 (Table-2). Serum leptin levels were also determined according to the level of proteinuria. Proteinuria of 1+ and 2+ were not found to be significant in relation to leptin but proteinuria of 3+ was found to be

significantly associated ($p<0.001$) with serum leptin 86.1 ± 5.58 (Table-3). When correlation coefficient was calculated between serum leptin and systolic and diastolic blood pressure in both the control and PE group, a significant positive correlation ($r=0.69$ & $r=0.76$) ($p<0.01$) was found between serum leptin and systolic and diastolic blood pressure in pre-eclamptic group as compared to control (Table-4).

Table-1: Serum leptin levels in normal pregnant women (controls) and in pre-eclamptic

Variables	Group A Control (n=45) Mean±SD	Group B Pre-eclamptic (n=45) Mean ±SD
Serum leptin (ng/ml)	26.8± 6.47	62.1± 23.8*

n= Number of subjects. * $p<0.001$ when compared to control.

Table-2: Serum leptin levels according to severity of pre-eclamptics

Variables	Pre-eclamptics (n=45)	
	Severe (n=17) Mean±S.D	Mild (n=28) Mean±S.D
Serum leptin (ng/ml)	85.7±13.3*	47.7±15.9

* $p<0.001$ when compared to control.

Table-3: Serum leptin level according to proteinuria in pre-eclampsia

Proteinuria	No. of subject	Serum Leptin (ng/ml) in Pre-eclampsia (n=45)
		Mean±SD
1+	18	52.1±4.23
2+	13	52.0±5.56
3+	11	86.1±5.58*
4+	3	77.6±13.38

*Significantly higher as compared to 1+ and 2+ ($p<0.001$)

Table-4: Correlation between blood pressure vs serum leptin in normal pregnant women (controls) and in pre-eclamptics

Blood pressure (mmHg)	Group A (control) (n=45) Serum leptin	Group B (Pre-eclamptic) (n=45) Serum leptin
	Systolic	$r= -0.14$
Diastolic	$r= -0.02$	$r= 0.76^*$

* $p<0.01$ statistically significant

DISCUSSION

PE is a pregnancy complication caused by factors released from a dysfunctional placenta.¹⁸ Delivery of the placenta results in clinical resolution and placenta is viewed as the essential organ in the development of PE.¹⁹ There is a possibility that leptin like several other placentally derived substances (e.g., Steroids, hormones, eiconasoids and cytokines) may be involved in the pathogenesis of PE.²⁰

In a longitudinal study done by Anim-Nyame in 2000, 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.²¹

Similarly Hytinantti in 2000 also reported that PE was associated with increased leptin concentration and that PE is an independent determinant of leptin levels.²² Under these circumstances, the current study was undertaken to compare and confirm the reported increase in serum leptin in PE and also to investigate the possibility of serum leptin being a marker of severity of PE.

The results of our study suggest that in pre-eclamptic patient in the third trimester of pregnancy, the levels of serum leptin are found to be elevated when compared to women with normal normotensive pregnancies. These findings are in agreement with the study done by Haugen¹⁹, Kocyigit²³ and Sebiha Ozkan²⁴, who studied leptin levels in PE. Moreover, the PE group in our study was divided in itself as mild and severe PE and the values of serum leptin were compared. Highly significant difference was found between two groups which was in concordance with the results of Atamer.¹⁷ We also relate serum leptin levels with proteinuria and found that as the protein increases, the leptin level also increases significantly. There could be several possible causes for elevated leptin levels.

Impaired renal function is a pathophysiological component of pre-eclampsia and the measured increase in plasma leptin concentration may reflect reduced renal clearance.²⁴ Also high leptin levels may be due to the possible haemoconcentration in pre-eclampsia caused by association of pre-eclampsia with reduced plasma volume.¹⁰

It has been suggested that BMI was responsible for the increase in maternal levels in pre-eclamptic women in several studies since adipose tissue is a source of leptin.^{12,25,26} In pregnancy however, the body mass index does not accurately reflect fat accrual because the fetus, the placenta, the amniotic fluid, increase plasma volume and available degree of extravascular fluid accumulation all increase maternal weight.¹⁰

Placental ischemia also explains rapid increase in leptin concentration during late third trimester in PE. Placental hypoperfusion produces local hypoxia which consequently augments leptin gene expression in the placenta¹². Finally there is evidence that inflammatory mediators increase plasma leptin concentration and in pre-eclampsia circulating concentration of the inflammatory cytokines such as TNF- α and IL-6 are increased.²⁷ Thus, there are several possible explanations for higher leptin concentrations in pregnancies complicated by pre-eclampsia. The exact mechanism underlying the increase in circulating leptin concentration in pre-eclampsia awaits further clarification.

CONCLUSION

From this comparative cross-sectional study, it is concluded that

- The levels of serum leptin are higher in established pre-eclampsia when compared to normotensive pregnant women and may contribute to endothelial dysfunction involved in the pathogenesis of pre-eclampsia.
- Also the increased levels of leptin in severe pre-eclamptic as compared to mild pre-eclamptics helps us to prove our point that leptin may serve as a marker of severity of pre-eclampsia.
- The cross-sectional design of this study prevents us from drawing conclusion as to what causes elevated leptin levels during pre-eclampsia. Our focus was to monitor the leptin concentration during pre-eclampsia as it may help in the prediction of the disease but the functional significance is still unclear.
- The mechanism responsible for this increase and the role played by leptin in the development of pre-eclampsia requires further study.

SUGGESTION

Determination of leptin levels during early pregnancy may prove useful as elevated leptin concentrations were observed even before the pre-eclampsia is clinically evident. It is therefore helpful in indicating the severity of disease in pregnancies destined to become complicated by pre-eclampsia. Thus leptin assay in early pregnancy will guide the clinician to take appropriate management steps for those patients who are at a risk of developing pre-eclampsia.

REFERENCES

1. Noris M, Perico N, Remuzzi G. Mechanism of disease: Pre-eclampsia. *Nat Clin Pract Nephrol* 2005;1(2):98-114.
2. National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Report of the NHBPEP Working Group on High Blood Pressure in Pregnancy. *Am J Obstet Gynecol* 2000;183:S1-S22.
3. Ramsay JE, Jamieson N, Greer IA and Sattar N. Paradoxical elevation in adiponectin concentrations in women with pre-eclampsia. *Hypertension* 2003;42:891-4.
4. Kaunitz AM, Hughes JM, Grimes DA, Smith JC, Rochat RW, Kafrisen ME. Causes of Maternal Mortality in the United States. *Obstet Gynecol* 1985;65:605-12.
5. James DK, Weiner CP, Steer PJ, Gonik B. Hypertension High risk pregnancy 3rd ed, Saunders, 2006; pp.722-97.
6. Walsh SW. Pre-eclampsia: An imbalance in placental prostacyclin and thromboxane production. *Am J Obstet Gynecol* 1985;152:335-40.
7. Roberts JM, Redman CWG. Pre-eclampsia: more than pregnancy-induced hypertension. *Lancet* 1993;341:1447-51.
8. Fruehwald-Schultes B, Peters A, Kern W *et al*. Serum leptin is associated with serum uric acid concentration in human. *Metab* 1999;48:677-80.
9. Gong DW, Bi S, Pratley RE, Weintraub BD. Genomic structure and promoter analysis of the human obese gene. *J Biol Chem* 1996;271:3971-4.

10. Mc Carthy JF, Misra Dn, Roberts JM. Maternal plasma leptin is increased in pre-eclampsia and positively correlates with fetal cord concentrations. *Am J Obstet Gynecol* 1999;180:731–6.
11. Bajoria R, Sooranna SR, Ward BS, Chatterjee R. Prospective function of placental leptin at maternal fetal interface. *Placenta* 2002;23:103–15.
12. Mise H, Sagawa N, Matsumoto T, Yura S, Nanno H, Itoh H, *et al.* Augmented placental production of leptin in pre-eclampsia: Possible involvement of placental hypoxia. *J Clin Endocrinol Metab* 1998;83:3225–29.
13. Lord, G.M., Matarese, G., Howard, JK. Baker RJ, Bloom SR, Lechler RI. Leptin modulate the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* 1998;27:897–901.
14. Yoneyama Y, Sawa R, Suzuki S, Miura A, Kobayashi H, Doi D, *et al.* Relation between adenosine deaminase activities and cytokine-producing T cells in women with pre-eclampsia. *Clin Biochem* 2002;35:303–6.
15. Rein DT, Schondorf T, Gohring UJ, Kurbacher CM, Pinto I, Breidenbach M, *et al.* Cytokine expression in peripheral blood lymphocytes indicates a switch to T_H17 cells in patients with pre-eclampsia. *J Reprod Immunol* 2002;54:133–42.
16. Bartha JL, Romero-Carmona R, Escobar-Llompert M, Comino-Delgado R. The relationships between leptin and inflammatory cytokines in women with pre-eclampsia. *Br J Obstet Gynecol* 2001;108:1272–1276.
17. Atamer Y, Erden AC, DeMIR B, Kolyigit Y, Atame A. The relationship between plasma levels of leptin and androgen in healthy and pre-eclamptic pregnant women. *Acta Obstet Gynecol Scand* 2004;83:425–30.
18. Singh HJ, Asiah AB, Aminah CR, Nila A. Raised leptin concentration in fetoplacental tissues from women with pre-eclampsia. *Hyperten Preg* 2005;24:191–9.
19. Haugen F, Ranheim T, harsem NK, Lips E, Staff AC, Drevon CA. Increased plasma levels of adipokines in pre-eclampsia: relationship to placenta and adipose tissue gene expression. *Am J Physiol Endocrinol Metab* 2006;290:E326–E333.
20. Williams MA, Haver PJ, Schwartz MW, Leisenring WM, King IB, Zingheim RW, *et al.* Pre-eclampsia disrupts the normal relationship between serum leptin concentrations and adiposity in pregnant women. *Paediatr Perinat Epidemiol* 1999;13:190–204.
21. Anim-Nyame N, Sooranna SR, Steer PJ, Johnson MR. Longitudinal analysis of maternal plasma leptin concentrations during normal pregnancy and pre-eclampsia. *Human Reproduction* 2000;15:2033–6.
22. Hytinen T, Koistinen HA, Koivisto VA, Karonen SL, Rutanen BM, Andersson S. Increased leptin concentration in preterm infants of pre-eclamptic mothers. *Arch Dis Child Fetal Neonat Ed* 2000;83:F13–F16.
23. Kocycit Y, Atamer Y, Atamer A, Tuzau A, Akkus Z. Changes in serum levels of leptin, cytokines and lipoprotein in pre-eclamptic and normotensive pregnant women. *Gynecol Endocrinol* 2004;19:267–73.
24. Sebiha O, Cemar TE, Riza M, Kilic A. Serum leptin levels in hypertensive disorder of pregnancy. *Eur J Obstet Gynecol Reprod Biol* 2005;120:158–163.
25. Kafalafula GE, Moodley J, Ojwang PS, Kagoro H. Leptin and pre-eclampsia in black African parturients. *Br J Obstet Gynecol* 2002;109(11):1256–61.
26. Solomon LJ, Benattar C, Audibert F, Fernandez H, Duyme M, Taieb J, Frydman R. Severe pre-eclampsia is associated with high inhibin A levels and normal leptin levels at 7 to 13 weeks into pregnancy. *Am J obstet Gynecol* 2003;189:1517–22.
27. Vince GS, Starkey PM, Austgulen R *et al.* Interleukin-6, tumour necrosis factor and soluble tumour necrosis factor receptors in women with pre-eclampsia. *Br J Obstet Gynecol* 1995;102:20–5.

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