

## Fasting insulin levels, lipid profile and proteinuria an index of cardiovascular risk in euglycemic preeclampsia

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### Abstract

Proteinuria has its value in pregnancy and cardiovascular risk assessment in diabetes, hypertension etc. Preeclampsia is subtypes of hypertensive disorder of pregnancy (HDP) with proteinuria complicating 7-10% of pregnancies in India. To evaluate the cardio metabolic risk factors as assessed by serum fasting insulin, lipids and its ratios in preeclampsia and to assess the relationship between proteinuria and insulin. Two groups; Healthy pregnant control (n=30) and preeclampsia (n=30). Statistical analysis was done by unpaired student t test with welch correction. Mean age of the pre-eclamptic mother was  $23 \pm 2.5$  SD and gestational period  $31.6 \pm 1.5$  weeks and average amount of protein lost in urine for 24 hours was 447.2 mg/day, there was significant increase in mean arterial BP in preeclampsia ( $p=0.000$ ), there was significant decrease in serum insulin levels but HOMA-IR was not significant. Serum ALP level, Total cholesterol, triacylglycerol (TAG), cholesterol / HDL ratio, triacylglycerol/ HDL ratio was significantly higher in proteinuric preeclamptic patients when compared to control ( $p= < 0.05$ ). HDL-C was significantly lower in protenuric preeclampsia when compared to control. There was no significant difference in the level of LDL between preeclampsia and control. There was an association between plasma insulin and proteinuria in preeclampsia ('r' value = 0.440; 'p' value = 0.015). Euglycemic proteinuric hypertensive disorders of pregnancy are at risk for cardiovascular diseases and estimation of fasting insulin and proteinuria can be useful as a marker.

**Key-words:** Preeclampsia, Proteinuria, lipid profile and insulin

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### Introduction

Estimation of protein in urine during pregnancy is vital and it an important indicator of maternal risk for obstetrics care providers [1]. Based

on the presence and absence of proteinuria, Hypertensive disorder of pregnancy (HDP) can be classified as preeclampsia and gestational hypertension together accounts for major cause of maternal mortality in complicating 5-10 % pregnancy worldwide. Preeclampsia which is characterized by the presence of proteinuria  $\geq 0.3$ mg/day or more, is more likely to have severe hypertension and maternal mortality than non proteinuric hypertensive disorder of pregnancy [2][3].

Proteinuria has its own diagnostic and prognostic significance in hypertension, diabetes mellitus and renal diseases.[4,5]. Studies have documented, the diagnostic use of urine micro albumin in pregnant women during early stages of the disease thereby preventing major complication of Hypertensive disorder of pregnancy (HDP).[6]

Insulin resistance (IR) is a state has been linked in proteinuria in patients with hypertension,

diabetes mellitus etc. [7-9]. But few studies documented conflicting report on (IR) in HDP. [10-12]. Recent study showed increasing incidence of cardiac vascular mortality among women. So the present study was designed to evaluate the cardio metabolic risk factors as assessed by insulin, lipids and its ratios in protenuric preeclampsia and to assess the relationship between proteinuria and plasma insulin.

### Material and methods

The study population includes pregnant women in and around Pondicherry. Present study was conducted after getting approval from institute ethical clearance and consent from the study participants who were recruited from Biochemistry collection Centre, antenatal ward and Outpatient department of Obstetrics. Study population included previously normotensive pregnant women who had raise in BP  $\geq 140/90$  mmHg in two separate occasions after 20 weeks of pregnancy were named as HDP and preeclampsia is subdivision with the presence of proteinuria (n= 30) (Modified from The National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy (NHBPEP) report) . Age and gestation matched non-proteinuria healthy primi-gravidae control (n= 30) were included. All the subjects were chosen between 25-30 years of age.

Exclusion criteria:

- a. Patients on other surgical & medical treatments

### Results

**Table 1:** Demographic characters, proteinuria, arterial blood pressure in preeclampsia subjects

Parameter	Control (n=30)	Preeclampsia (n=30) $\geq 300$ mg /day	P value
Age (Yrs)	23 $\pm$ 2.2	23 $\pm$ 2.5	NS
Gestational age (Wks)	31.8 $\pm$ 1.6	31.6 $\pm$ 1.5	NS
Proteinuria (mg/day)	00.00 $\pm$ 00	447.2 $\pm$ 167.02	---
MAP (mm Hg)	89 $\pm$ 3	110.33 $\pm$ 7.6	0.000
SBP (mm Hg)	112 $\pm$ 6	143 $\pm$ 13*	<0.0001
DBP (mm Hg)	77 $\pm$ 4	94 $\pm$ 7*	<0.0001

Data presented are mean  $\pm$  SD. Analysis of data was done by unpaired student t test with welch correction. The \*depicts comparison with Control group.\*p < 0.05. SBP- Systolic blood pressure, DBP- Diastolic blood pressure, MAP- mean arterial blood pressure , NS= not significant .

- b. Hypertension existing before pregnancy
- c. The pregnant women with heart disease, bronchial asthma and overt diabetes mellitus.
- d. Patients with gestational diabetes mellitus

Three ml of fasting blood and 24 hour urine samples were obtained from the study subjects. Samples were collected with preservative under strict aseptic precaution. Quantification of urine protein was done using IFCC approved turbidometric method. Serum was obtained after centrifuging samples at 3000 rpm (Remi-clinical centrifuge) for biochemical analysis of fasting lipid profile and hepatobiliary enzymes in fully automated clinical chemistry analyzer [Randox-Imola] Randox laboratories limited, Crumlin, UK, reagent kits were procured from Agappe diagnostics Kerala –India. Insulin resistance calculated using Homeostasis model assessment of insulin resistance HOMA –IR formula by measuring fasting glucose (GOD-POD method and fasting insulin by immunoradiometric assay (IRMA) kit from (IMMUNOTECH- Prague, Czech republic).  $HOMA-IR = [\text{fasting insulin } (\mu\text{U/ml}) \times \text{glucose (mmol/dl)}] / 22.5$ .

**Statistical Analysis:** The data is presented as mean and standard deviation and analyzed by using student’s t test. Pearson’s test was used for correlation analysis. A p value less than 0.05 were considered as the level of significance for all the tests. All statistical analysis was performed using SPSS version 17.

As depicted in Table 1. Gestational period and mean age between study groups were matched without any significant differences between them. There was a significant increase Systolic, Diastolic blood pressure and Mean Arterial Pressure in protenuric preeclampsia when compared to control.

**Table 2:** Serum fasting insulin level, glucose level, insulin Resistance and lipid alterations in preeclampsia subjects

PARAMETER	Control (n=30)	Preeclampsia ≥ 300 mg /day	P value
Triacylglycerol (mg/dl)	171 ± 58.7	223 ± 78.8*	0.005
Cholesterol (mg/dl)	196 ± 46.4	224 ± 70.3 *	0.030
HDL-C (mg/dl)	46 ± 6.6	32 ± 4.4*	< 0.000
LDL-C (mg/dl)	115 ± 42.1	147 ± 66	0.014
Cholesterol : HDL	4.3±1.1	7.07 ±2.5*	< 0.0001
Triacylglycerol: HDL	3.7±1.4	6.96 ±2.6*	< 0.0001
Insulin (µIU/L)	19.06± 0.4	16.9± 0.5*	< 0.0001
Fasting blood glucose (mg/dl)	70 ± 9.6	72.6±15.6	NS
HOMA – IR	4.26± 4.2	2.8±3.2	NS

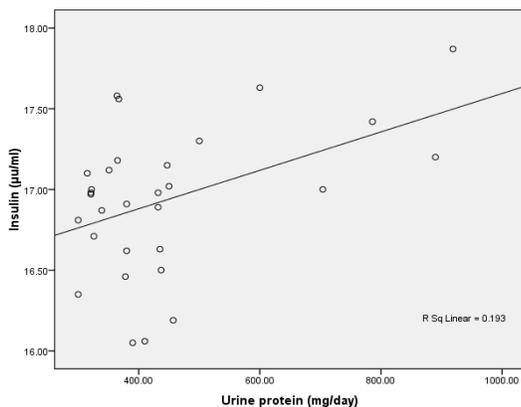
\* p= < compared to controls, NS =not significant. Values are expressed as mean ±SD.

Serum triacylglycerol (TAG) was significantly higher in proteinuric preeclampsia than control (p= <0.05). Total cholesterol level was significantly higher in protenuric preeclampsia when compared to control. HDL-C was significantly lower in protenuric preeclampsia when compared with control. There was no significant difference in the level of LDL-C between control and study group.

Cholesterol / HDL ratio; triacylglycerol/ HDL ratio was significantly higher in proteinuric preeclampsia when compared to control (p= < 0.05). There was no significant alteration in fasting blood glucose and insulin resistance between them. There was a tendency for reduced insulin resistance in proteinuria preeclampsia subjects, when compared control. There was a significant increased fasting level of insulin in proteinuria preeclampsia subjects when compared to control. There was a positive correlation observed between proteinuria and fasting insulin level in proteinuria preeclampsia subjects. (r=0.440 p= 0.015) as depicted in Figure 1.

**Figure 1:** Relationship between plasma insulin and urinary protein in preeclampsia;

‘r’ value = 0.440; ‘p’ value = 0.015 ; p < 0.05 considered significant



**Table 3: Serum levels of hepatic enzymes**

PARAMETER	CONTROL (n=30)	PREECLAMPSIA ≥ 300 mg /day (n=30)	P value
AST (IU/L)	31.0 ± 16.2	48.1 ± 68.9	NS
ALT(IU/L)	33.2 ± 19.2	36.6 ± 58	NS
ALP (IU/L)	119 ± 72	216 ± 73.8*	<0.0001

Data presented are mean ± SD. Analysis of data was done by unpaired student t test with welch correction. The \*depicts comparison with Control group.\*p < 0.05. NS = not significant.

ALP level was significantly higher in proteinuric preeclampsia when compared to control. And other enzymes AST, ALT showed no significant alteration in protenuric preeclampsia.

### Discussion

The mean amount of protein lost in urine for 24 hours was 447.2 mg/day in preeclampsia with mean age  $23 \pm 2.5$  years and gestational age  $31.6 \pm 1.5$  weeks. With change in the lifestyle the incidence of cardiovascular diseases are increasing in developing countries like India which is not only diabetic capital but also changing capital for cardiovascular diseases as well. With vague obscure cause on preeclampsia, we hypothesized that proteinuria in prompting the biochemical event through Insulin.

### Insulin resistance in preeclampsia

In our study, insulin resistance as assessed by HOMA-IR formulae was not significantly higher in preeclampsia when compared to control suggesting no insulin resistance in preeclampsia. Insulin resistance is the condition where there is impaired ability of the insulin to stimulate the uptake and disposal of glucose by muscle [9]. Previous study has shown that there was considerably higher levels of insulin resistance in pregnant control than non-pregnant especially during last trimester of normal pregnancy [10]. Several studies have documented varying reports on insulin resistance among hypertensive disorder of pregnancy. In concordance with our result recent study conducted by Teimoori et al, 2009 in preeclampsia showed no insulin resistance in preeclampsia.(Table 2) Though, literature documents insulin resistance in HDP, still there are inconsistent reports on the occurrences of preeclampsia in relation to body mass index, dyslipidemia and metabolic syndrome in different geographical regions [10-12]. Recent report documents that not only pre-pregnancy weight determine maternal complications but also weight gain during the pregnancy can also signify maternal complications like preeclampsia commonly detected by the presence of significant proteinuria [12]. Moreover, some author considered proteinuria HDP(Preeclampsia) as different entity from other groups of HDP with different pathogenesis in causing endothelial dysfunction [13][8]. The studies documented on Insulin resistance in preeclampsia are listed in Table 4.

Study	Conclusion of the study
Caruso A et al 1999	Data do not support an association between insulin resistance syndrome and hypertension in pregnant women with preeclampsia and chronic hypertension. [8]
Salamalekis, E et al 2005	Preeclampsia is not associated with hyperinsulinaemia and/or insulin resistance, in either the fasting or the postprandial state. [14]
Teimoori B et al 2008	No relevant observed relation between severe preeclampsia and resistance to insulin. [15]
Romero Gutiérrez G et al 2003	Insulin resistance assessed in the third trimester of the pregnancy did not have association with the pregnancy induced hypertension [16]
Jacobson S et al 1994	Postpartum Blood Pressure and Insulin Sensitivity in African-American Women With Recent Preeclampsia suggests that the elevation in postpartum systolic blood pressure may reflect a persistent abnormality of blood pressure homeostasis which is not associated with insulin resistance. [17]
Sharma A et al 2002.	The pathophysiology of pre-eclampsia is not associated with insulin resistance. [18]
Masuyama H et al 2011	Insulin resistance (HOMA-IR) was there in overweight patients with late-onset preeclampsia were significantly higher than in overweight controls carrying normal pregnancies and in normal weight women with late-onset preeclampsia. [19]

As India is considered as the diabetic capital of the world, the insulin resistance among the Indian is slightly higher even in general population. Study by Trikudanathan S et al 2013 documented increased incidence of visceral adiposity among south Asian due genetic, nutritional and environmental factors could be contributing to the epidemic of high insulin resistance. [20] Further any form of stress like pregnancy could be further enhancing the level of insulin resistance among the Indian women as observed among the normal pregnant in our study. Even though study have documented insulin resistance in normal pregnancy due to hormonal change reason remains vague [10]. One of the major limitations in our study is involvement of non-pregnant control which could have fostered additional value. Experimental studies have documented high insulin level can independently increases blood pressure irrespective of placental factors[21]. In our study when compared to controls there was significant higher mean arterial blood pressure (Table 1) there was an association between plasma insulin level and proteinuria suggesting insulin effect on renal glomerular capillary endothelium.[22], Study have also documented high grade proteinuria is common and always reversible after pregnancy and it's considered a risk factor for cardiovascular diseases and mortality [23, 24, 25]. Further studies have documented the role of altered lipid metabolism during early pregnancy is considered risk for potential preeclampsia due to endothelial dysfunctions in diseases causation and future cardiovascular risk. [20-22]. Yet another study documents potential atherogenic risk mediated by small sized LDLc .So we tried to find LDL size indirectly, by calculating ratios like Total Cholesterol/HDL ratio and triacylglycerol/HDL ratio, both the ratios were found to be higher when compared to control studies have also documented ability of small sized LDL to cross vascular endothelium suggesting us unholy increment in Total Cholesterol/HDL ratio and triacylglycerol/HDL ratio and future risk for the development of complications [26, 27].

In our study liver enzymes were normal as depicted in table 3. Suggesting normal liver function without severity like HELLP syndrome, with altered insulin and lipid status. However, increased alkaline phosphatase activity in preeclampsia suggesting active milieu in placenta excluding bone and intestinal cause. Recent study from our department by Reeta R et al have documented diagnostic utility of serum placental alkaline phosphatase (PLAP) activity in hypertensive disorder of pregnancy (yet to be published). Study by Leitner K et al have documented PLAP as a morphological marker to differentiate syncytiotrophoblasts from cytotrophoblasts or as a marker enzyme for placental brush-border membranes where disease pathology could have originated [28].

### Conclusion

So, we conclude that there is dyslipidemia and a weak association between fasting insulin and proteinuria which can cause endothelial injury therein potentiate cardiovascular risk in euglycemic proteinuric hypertensive disorder of pregnancy.

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