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Altered Placental Insulin Signaling in the Absence of Systemic Insulin Resistance in Hypertensive Disorders of Pregnancy among South Indian Tamil Population

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Abstract

A cross sectional study was conducted to explore the possible derangements in placental insulin signaling and also to investigate the presence or absence of maternal systemic insulin resistance in different types of hypertensive disorders of pregnancy (HDP).

Study Design: Healthy pregnant women (n=32) and pregnant women with gestational hypertension (GH) (n=18), late onset Pre-eclampsia (LP) (n=18) and early onset Pre-eclampsia (EP) (n=19) were recruited for the study. Maternal venous blood and placental samples were collected from the study subjects at 35±7 weeks of gestation. Insulin resistance parameters such as fasting glucose, fasting insulin, homeostatic model assessment of insulin resistance, beta cell function and insulin sensitivity and glycated hemoglobin were estimated in the maternal venous blood samples. Placental protein expressions of insulin signaling molecules were analyzed by western blotting method.

Results: Maternal venous blood insulin resistance parameters were not significantly different between the study groups. However, placental content of phospho insulin receptor beta was significantly decreased in GH, LP and EP groups in comparison to controls. We also found a significant decrease in placental protein expression of phosphatidyl inositol 3-kinase p85 alpha in LP group when compared to other groups.

Keywords: Placenta; Insulin signaling; Gestational hypertension; Late onset Pre-eclampsia; Early onset Pre-eclampsia.

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INTRODUCTION

Hypertensive disorders of pregnancy affects 15% of pregnancies and is the most common health problem for women and their babies.[1] It is one of the major reasons for neonatal, fetal and maternal morbidity and mortality.[2] In the year 2000, maximum number of maternal mortality in the world (1,36,000), were reported in India.[3] Hypertensive disorders of pregnancy (HDP) rank

second to hemorrhage as a leading direct cause of maternal mortality and contributes to 14% of the maternal deaths worldwide.[4] Incidence of Pre-eclampsia in developing countries is reported to be seven times higher than the developed countries.[5]

While complications in gestational hypertension are less severe[6], Pre-eclampsia is associated with multiple organ insufficiency and risk for future cardiovascular diseases in mother and offspring.[6] To date, the only effective cure of HDP is termination of the pregnancy and expulsion of the placenta.[7]

Pathophysiology of HDP is still not understood well.[7] It is widely accepted that gestational hypertension, early and late onset Pre-eclampsia have different pathologies and should be considered as different disorders as there are differences in clinical manifestation, prognosis, complications and maternal and perinatal outcomes.[8,9] Among the different types of HDP, Pre-eclampsia is best studied and studies on gestational hypertension are very limited. Since the idea of early and late onset Pre-eclampsia is fairly young, only few studies have individually assessed these categories of Pre-eclampsia.

Placenta is believed to have a significant contribution to the pathogenesis of HDP, since only after the expulsion of the placenta the disease is completely resolved.[10] Glucose is an importantenergy source for the fetus and the placenta.[11] Glucose is required for the fetal growth and for the metabolic needs of the placenta.[11] Though maternal insulin cannot cross the placenta, it binds to insulin receptors present in syncytiotrophoblast membrane of the placenta and initiates the insulin signaling cascade in the placenta.[12] Insulin signaling pathway[13] is depicted in Fig. 1.

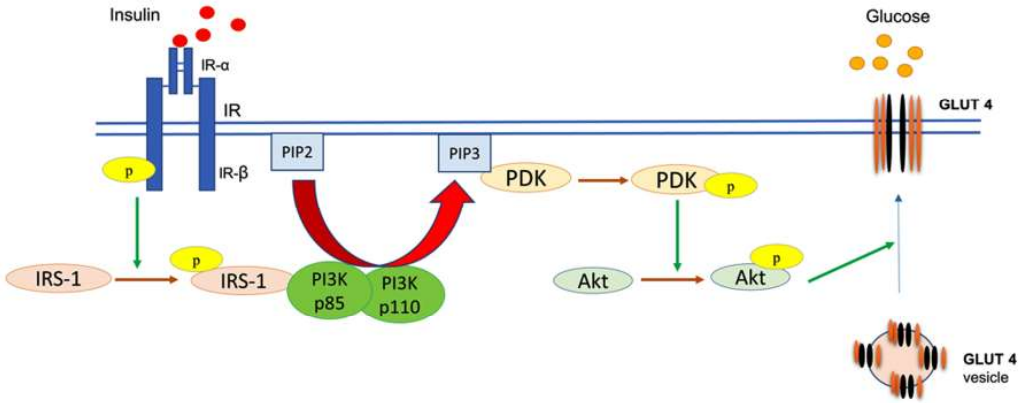

Fig. 1: Insulin signalling cascade. IR: insulin receptor; IRS-1: insulin receptor substrate-1; PI3K: phosphatidyl inositol 3 kinase; PIP2: phosphatidyl inositol (4,5) bisphosphate; PIP3: phosphatidyl inositol (3,4,5) triphosphate; PDK: 3-phospho inositide dependent protein kinase; GLUT-4: glucose transporter 4. p: phosphorylation. When insulin binds to IR-α subunit, autophosphorylation of a number of tyrosine residues of the beta subunit occurs. These phospho tyrosine residues are recognized by insulin receptor substrate and results in subsequent phosphorylation of its tyrosine residues. Some of these residues are recognized by PI3K subunit, p85 and then, p110 subunit of PI3K phosphorylates PIP2 and converts it to PIP3. Phosphatidyl inositol triphosphate recruits Akt and PDK-1 to the cell membrane and enables PDK-1 to activate Akt. Activated Akt translocates glucose transporters from its intracellular storage to the cell membrane and increases the uptake of glucose into the cell.

Placental insulin resistance is evolving as one of the key mechanisms in the pathogenesis of Pre-eclampsia.[14] Serine phosphorylation of IRS-1 has been reported to be higher in preeclamptic placenta in comparison to controls.[14] However, only few studies have traced the signal transduction pathway of insulin in the placenta of women with HDP.[14–16] Further, while some studies have reported systemic insulin resistance in Pre-eclampsia[17–19], other studies have reported its absence.[20,21]

We explored the possible derangements in placental insulin signaling and also investigated the presence or absence of maternal systemic insulin resistance in different types of HDP among South Indian Tamil population. The ultimate aim of research in this field is to help obstetricians to effectively manage the pregnant women with hypertensive disorders in order to avoid/delay

preterm termination of pregnancy and to improve the fetal outcome.

MATERIALS AND METHODS

Study Participants

This study was approved by institute ethics committee (Human studies), JIPMER, Puducherry (JIP/IEC/2015/14/547). Pregnant women aged between 18 to 35 years were recruited for the study after obtaining informed consent. Study subjects were divided into four categories (control, gestational hypertension, late onset Pre-eclampsia and early onset Pre-eclampsia) based on American College of Obstetricians and Gynecologists guidelines 2013.[22,23]

Inclusion Criteria

Healthy pregnant women who did not have present or past history of hypertensive disorders and any of the complications of pregnancy were recruited into the control (C) group. Systolic blood pressure (SBP) of ≥ 140 mmHg and/or diastolic blood pressure (DBP) of ≥ 90 mmHg determined on two occasions at least four hours apart was defined as hypertension.[22] Pregnant women who developed hypertension first after twenty weeks of gestation without proteinuria or systemic findings were included in gestational hypertension (GH) group.[22] Pregnant women who developed hypertension after 34 weeks of gestation with one or more of the systemic findings (impaired liver function, new onset of cerebral or visual disturbances, thrombocytopenia, proteinuria, pulmonary edema and new development of impaired renal function) were recruited into late onset Pre-eclampsia (LP) group.[22,23] Pregnant women who developed hypertension between 20 and 33+6 weeks of gestation with one or more systemic findings were included in early onset Pre-eclampsia (EP) group.[22,23]

Exclusion Criteria

Pregnant women with gestational diabetes mellitus, renal diseases, hypothyroidism, Pre-eclampsia super imposed on chronic hypertension, infections in current pregnancy and chronic hypertension were excluded from the study.

Sample Collection

Women from South India were recruited for the study. Cases and controls were recruited from Obstetrics and Gynecology department, JIPMER. Maternal fasting venous blood samples were collected from 87 study participants (C-32, GH-18, LP-18 and EP-19) at 35±7 weeks of gestation. They were followed till delivery and 20 placental samples were collected immediately after delivery (C-5, GH-5, LP-5 and EP-5). From the subjects recruited, placentas were collected based on convenience sampling method. A central lobule (cotyledon) of placenta (maternal side) was collected and washed with ice cold phosphate buffer saline and dissected into small pieces to remove calcium deposits and visible connective tissue. Placental protein expressions of insulin signaling molecules were analyzed infive samples in each group.

Estimation of Blood Insulin Resistance Parameters

Fasting glucose level in maternal serum was estimated using Olympus AU400 fully automated Clinical Chemistry Analyzer. Fasting insulin level was assessed by commercially available ELISA kit (DIA source Immuno Assays, Belgium). Homeostatic model assessment (HOMA) of insulin sensitivity, insulin resistance and beta cell function were calculated by HOMA 2 calculator version 2.2.3 (University of Oxford).[24] Glycated hemoglobin (HbA1c) level was estimated by HPL Cmethod. For the estimation of glycated hemoglobin, maternal venous blood samples were collected in vials containing Ethylenediamine Tetraacetic Acid (EDTA) as the anticoagulant.

Western Blotting Analysis of Placental protein Expression

Placental samples were homogenized in RIPA buffer using Dounce homogenizer. Proteins were separated by SDS-PAGE. Proteins (50 ug each) were loaded into the wells. Separated proteins were transferred on tonitrocellulose membrane. Blocking of membranes was done with 5% bovine serum albuminor non-fat milk powder. Then, membranes were incubated overnight at 4° C with primary antibodies. Primary antibodies used were insulin receptor beta (IR-β); phospho (Y1361) insulin receptor beta (pIR-β); IRS-1; glucose transporter-4 (GLUT-4) [Cell Signaling Technology, USA], phosphatidyl inositol 3-kinase p85 alpha (PI3K p85-α) [Novus Biologicals, CO, USA], Akt; beta actin [Thermo Fischer Scientific, Waltham, MA, USA] and 3-phospho inositide dependent protein kinase-1 (PDK-1) [Santa Cruz Biotechnology, USA].

After primary antibody incubation, membranes wereincubated with species specific secondary antibody (HRP goat Anti-Mouse IgG or HRP goat

Anti-Rabbit IgG) (ABclonal Technology, USA) for two hours at room temperature. The bands obtained by the binding of antibody were detected using enhanced chemiluminescence method. The band density was quantified using Image Lab software version 6.0.1 (Bio-Rad, USA). In the analysis, the density of the control band was considered to be one and the density of the test bands were analyzed relative to the control band. The band density was expressed in terms of relative units.

Statistical Analyses

The values are expressed as mean ± SD/SEM for parametric data and median (interquartile range) for non-parametric data. To compare the parametric data between the study groups, One-way ANOVA with Tukey's multiple comparison test was used and for non-parametric data Kruskal-Wallis test with Dunn-Bonferroni post hoc test were used. A 'p' value less than 0.05 was considered as statistically significant. For statistical analysis, SPSS software (version 19.0; SPSS Inc., Chicago, IL, USA) and Graph Pad Prism 5 software for windows (Graph Pad Software, San Diego, CA) were used.

RESULTS

Baseline Characteristics of the Study Participants

There was no significant difference in the age between the study groups (Table 1). Systolic and diastolic blood pressure were significantly increased in all types of HDP groups in comparison to control group (Table 1). Time of onset of hypertension (TOH) was significantly earlier in EP women when compared to GH and LP women (Table 1). Also, gestational age at delivery in EP women was significantly lesser when compared to rest of the groups (Table 1). Birth weight of babies born to early onset preeclamptic women were also significantly reduced when compared to other groups (Table 1).

Table 1: Baseline characteristics of the study subjects

	C (n=32)	GH (n=18)	LP (n=18)	EP (n=19)
Age (years)	24.72±3.67	24.94±5.16	23.94±3.35	26.37±4.02
SBP (mm/Hg)[x]	110.5±9.44	140.78±9.0[aaa]	154.39±12.93[aaab]	163.68±21.85[aaabbb]
DBP (mm/Hg)[x]	71.63±8.21	92.0±6.36[aaa]	100.17±12.17[aaa]	100±11.24[aaa]
TOH (days)	-	265.39±13.76	260.50±14.91	205.89±22.32[bbbccc]
GA (days)	276.81±8.87	269±12.39	268.89±12.01	223.84±22.7[aaabbbccc]
BW (kg)	2.79±0.36	2.70±0.57	2.40±0.62	1.51±0.67[aaabbbccc]

Values are expressed as mean ± SD. The differences between groups were analysed usingone-way analyses of variance (one-way ANOVA) with Tukey's multiple comparison test. xHighest blood pressure recorded during the hospital stay. aaa: p value <0.001 in comparison to control; b: p value <0.05 in comparison to gestational hypertension; bbb: p value <0.001 in comparison to gestational hypertension; ccc: p value <0.001 in comparison to late onset Pre-eclampsia. C: control; GH: gestational hypertension; LP: late onset Pre-eclampsia; EP: early onset Pre-eclampsia; SBP: systolic blood pressure; DBP: diastolic blood pressure; TOH: time of onset of hypertension; GA: gestational age atdelivery, BW: birth weight of baby.

Maternal insulin Resistance Parameters in HDP

Maternal venous bloodfasting glucose, fasting insulin, HOMA2 beta cell function (HOMA2-% β), HOMA2 insulin sensitivity (HOMA2-%S), HOMA2 insulin resistance (HOMA-IR) and HbA1c levels did notdiffer significantly among the study groups (Table 2).

Table 2: Maternal blood insulin resistance parametersin patients with hypertensive disorders of pregnancy

	C (n=32)	GH (n=18)	LP (n=18)	EP (n=19)
Fasting glucose (mg/dL)	78.56 ± 8.65	72.28 ± 9.23	71.72 ± 10.33	74.63 ± 10.40
Fasting insulin (μIU/mL)[x]	9.48(8.50-14.25)	10.81(8.84-12.92)	10.04(9.12-13.58)	10.04(8.49-12.79)
HOMA2-%B	169.2 ± 36.3	198.4 ± 60.9	205.4 ± 67.5	182.8 ± 68.3
HOMA2-%S	73.2 ± 19.2	77.7 ± 17.9	77.5 ± 19.2	81.8 ± 20.8
HOMA2-IR[x]	1.31(1.15-1.76)	1.32(1.10-1.59)	1.19(1.12-1.71)	1.25(1.04-1.47)
HbA1c(%)[y]	5.37 ± 0.36	5.42 ± 0.31	5.50 ± 0.27	5.41 ± 0.35

Values are expressed as mean ± SD.x values are expressed as median (interquartile range). The differences between the groups were not statistically significant.y Sample size for HbA1c is C- 20; GH-16; LP-13 and EP-19. HOMA: homeostatic model assessment; HOMA2-%B: HOMAbeta cell function; HOMA2-%S: HOMA insulin sensitivity; HOMA-IR: HOMA insulin resistance; HbA1c:

glycated hemoglobin. C: control; GH: gestational hypertension; LP: late onset Pre-eclampsia; EP: early onset Pre-eclampsia.

Placental Insulin Signaling in HDP

Placental protein expression of IR-β (Fig. 2A) was significantly higher in LP group in comparison to C and EP groups. However, phospho IR-β (active form) content was significantly decreased in GH, LP and EP groups in comparison to controls (Fig. 2B).

In addition, protein expression of IRS-1 was not significantly different in cases in comparison to controls (Fig. 2C). Whereas, Protein expression of PI3K p85-α was significantly decreased in LP group in comparison to rest of the groups (Fig. 2D).

Further, placental protein expressions of PDK-1

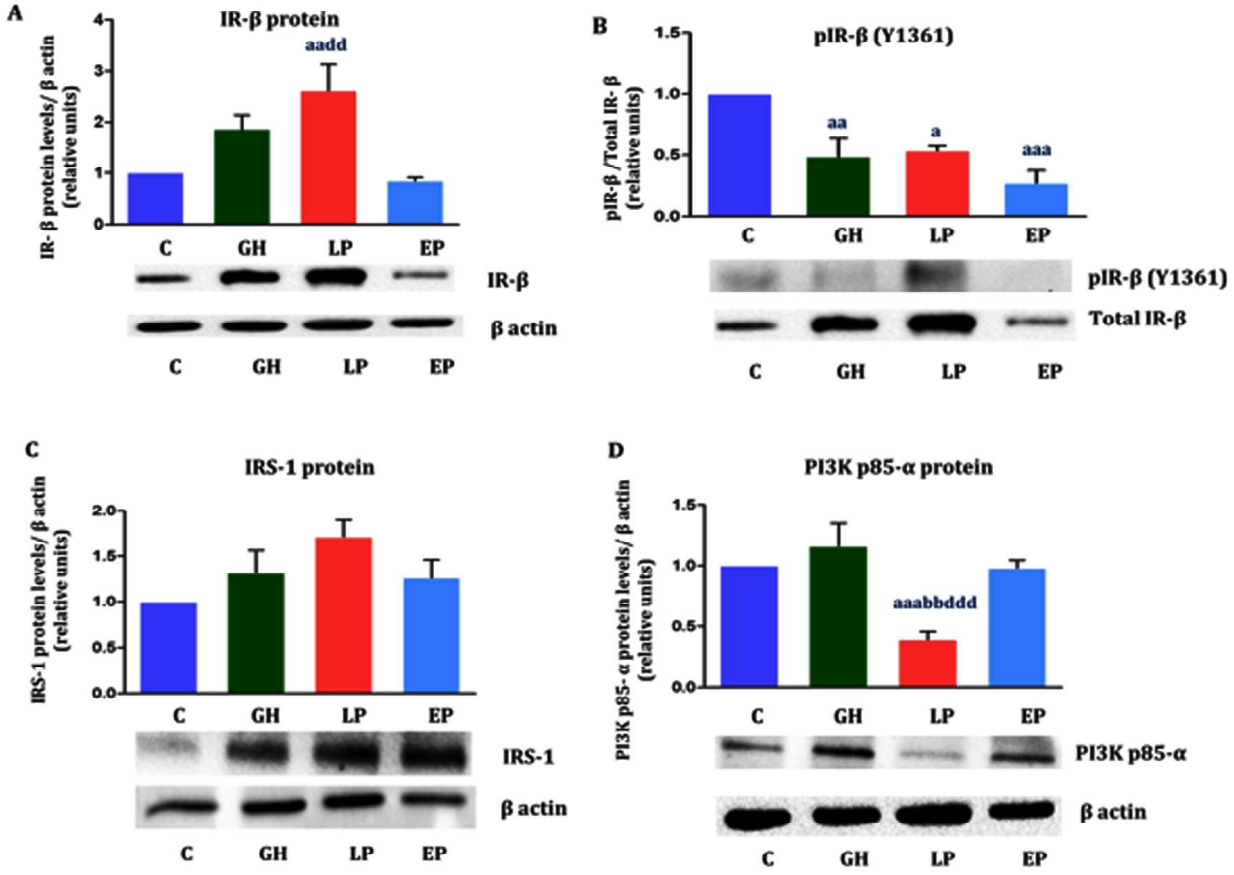

Fig. 2: Placental expressions of (A) insulin receptor beta (IR-β) protein (B) phospho insulin receptor beta (pIR-β) content (C) insulin receptor substrate-1 (IRS-1) protein and (D) phosphatidyl inositol 3-kinase p85 alpha (PI3K p85-α) protein in hypertensive disorders of pregnancy. Values are expressed as mean ± SEM of five samples. The differences between groups were analysed using one-way analyses of variance (one-way ANOVA) with Tukey's multiple comparison test. a: p value <0.05 in comparison to control; aa: p value <0.01 in comparison to control; aaa: p value <0.001 in comparison to control; bb: p value <0.01 in comparison to gestational hypertension; dd: p value <0.01 in comparison to early onset Pre-eclampsia; ddd: p value <0.001 in comparison to early onset Pre-eclampsia. C: control; GH: gestational hypertension; LP: late onset Pre-eclampsia; EP: early onset Pre-eclampsia.

and Akt were significantly increased only in GH group when compared to C, LP and EP groups (Fig. 3A & 3B). Similarly, protein expression of GLUT-4 was found to be significantly higher in Pre-eclampsia (early and late) groups when compared to control and GH groups (Fig. 3C).

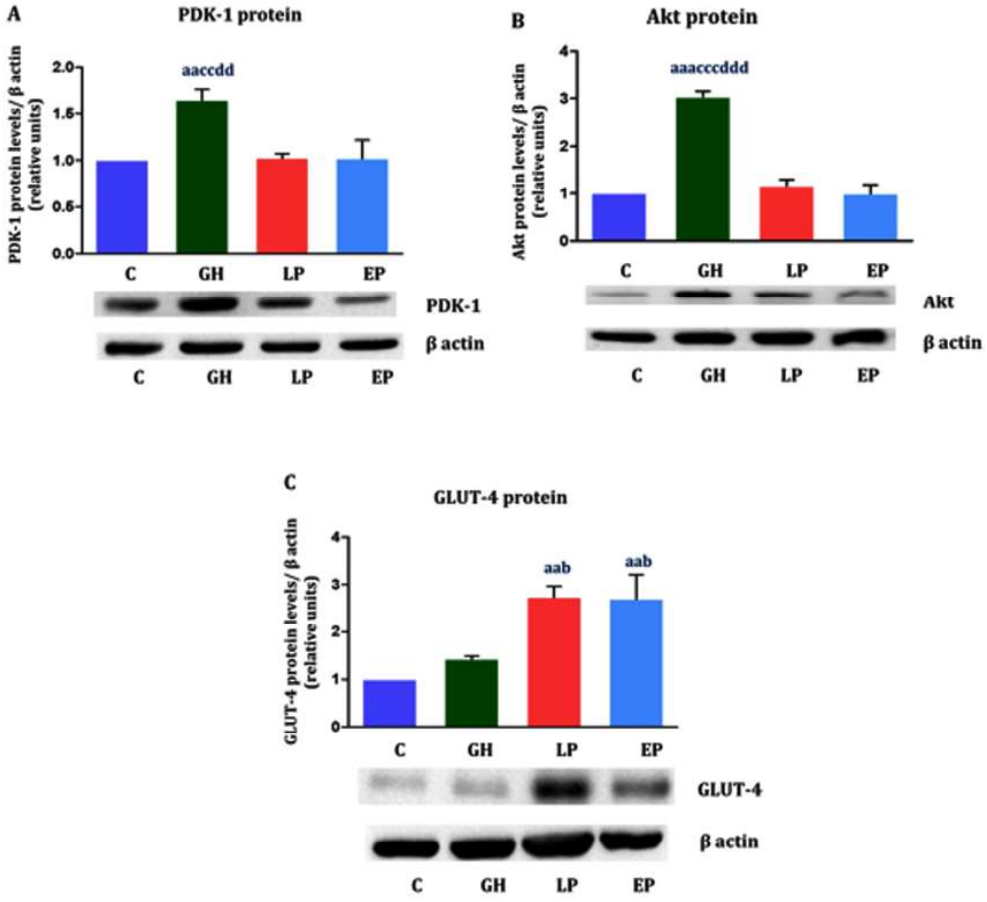

Fig. 3: Placental protein expressions of (A) 3-phospho inositide dependent protein kinase-1 (PDK-1) (B) Akt and (C) glucose transporter-4 (GLUT-4) in hypertensive disorders of pregnancy. Values are expressed as mean ± SEM of five samples. The differences between groups were analysed using one-way ANOVA with Tukey's multiple comparison test. aa: p value <0.01 in comparison to control; aaa: p value <0.001 in comparison to control; b: p value <0.05 in comparison to gestational hypertension; cc: p value <0.01 in comparison to late onset Pre-eclampsia; ccc: p value <0.001 in comparison to late onset Pre-eclampsia; dd: p value <0.01 in comparison to early onset Pre-eclampsia; ddd: p value <0.001 in comparison to early onset Pre-eclampsia. C: control; GH: gestational hypertension; LP: late onset Pre-eclampsia; EP: early onset Pre-eclampsia.

DISCUSSION

Insulin resistance is a state where cells cannot respond normally to the normal circulating levels of hormone insulin.[25] While some studies have reported the presence of systemic insulin resistance in Pre-eclampsia[17–19], others have reported its absence.[20,21] Our results also show the absence of systemic insulin resistance in all the HDP groups studied. The absence of systemic impaired glucose tolerance observed at present when compared to previous reports may be attributed to the improvements in the management especially by the early administration of magnesium sulphate.

Placental insulin resistance is evolving as a crucial mechanism in the HDP development.[14] A defect in signal transduction of the hormone insulin is defined as insulin resistance.[26] Studies on possible molecular mechanism of placental insulin resistance in HDP are very limited.[14–16]

In the present study, we found that phospho IR-β (active form) content was significantly decreased in all three types of HDP groups in comparison to controls (Fig. 2B). Also, PI3K p85 alphaprotein expression was significantly decreased only in LP group when compared to controls (Fig. 2D). Our results suggest the presence of placental insulin resistance in GH, LP and EP groups. In contrast to our findings, a previous study has documented a significant increase in phospho IR-β and PI3K p85-α in preeclamptic placenta in comparison to

control placenta.[14] However, there are no previous studies available on placental phospho IR-β content and PI3K p85-α protein expression in GH, LP and EP.

In addition, we also found a significantly increasedprotein expression of IR-β in LP group when compared to control and EP groups (Fig. 2A). These increased expressions could be the result of body's own compensatory response to improve the placental insulin resistance present in LP group. In contrary to our finding, previous study has documented an unchanged protein expression of IR-β in preeclamptic placenta when compared to control placenta.[14] However, there are no studies available in the literature on IR-βprotein expression in different types of HDP such as GH, LP and EP.

Furthermore, we found an increased expressions of PDK-1 and Akt in GH group when compared to controls and late and early onset Pre-eclampsia (Fig. 3A & 3B). These increase in expressions also could be a body's compensatory mechanism to tackle placental insulin resistance in gestational hypertension. There are no previous studies available on placental protein expression of PDK-1 in Pre-eclampsia or GH, LP and EP.

In addition, protein expression of GLUT-4 was found to be significantly increased in late and early onset Pre-eclampsia (Fig. 3C). There are no previous studies available on GLUT-4 expression in preeclamptic women. To the best of our knowledge, ours is the first study to report GLUT-4 expression in GH, LP and EP groups.

Moreover, we found an increased expression of IRS-1 in GH, LP and EP groups when compared to controls, however, the increase was not statistically significant (Fig. 2C). Previous studies have reported an unchanged placental protein expression of IRS-1 in Pre-eclampsia[14] and gestational hypertension[27] when compared to control pregnant women. However, there are no studies available on placental protein expression of IRS-1 in early and late onset Pre-eclampsia.

The pathogenesis of HDP is not completely understood till date. The findings of the present study provide better insight into the pathogenesis of different types of HDP. Our study explains the molecular basis of placental insulin resistance in different types of HDP even when there was no systemic insulin resistance and impaired glucose tolerance. However, further studies are needed to confirm the findings of the present study with larger sample size.

CONCLUSION

This is the first study to explore the molecular basis of placental insulin resistance in different types of HDP. Although insulin resistance was not observed in maternal circulation, placental expressions of insulin signaling molecules were significantly altered in gestational hypertension, late onset Pre-eclampsia and early onset Pre-eclampsia. Addressing these derangements might result in better maternal and fetal outcomes in HDP. This study would assist in the development of novel drug targets for better management.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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