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The role of novel hormone asprosin in insulin resistance during preeclampsia

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Abstract: Preeclampsia, a multisystemic syndrome of unknown etiology is characterized by hypertension and proteinuria after 20 weeks of gestation. Metabolic alterations causing insulin resistance and hyperinsulinemia are the prominent factors of preeclampsia. A novel hormone asprosin was discovered to be increased in humans with pathological conditions related to insulin resistance. This study aimed to find relationship between insulin resistance related-hormone asprosin and preeclampsia. A comparative cross-sectional study was conducted on 21 preeclamptic pregnant mothers and 21 healthy pregnant mothers. Samples were taken from mothers at the time of delivery and were processed for estimation of asprosin, insulin and glucose hormones. Data was analysed using SPSS 21. Normality of the data was checked and Independent t-test was applied. A p-value of ≤0.05 was considered significant. Levels of asprosin, insulin, glucose and HOMA-IR index were significantly elevated in preeclamptic pregnant mothers when compared with healthy pregnant mothers with p-values 0.000, 0.003, 0.036 and 0.002 respectively, suggesting potential role of asprosin in insulin resistance during preeclampsia.

Keywords: Preeclampsia, insulin resistance, asprosin, insulin, HOMA-IR.

INTRODUCTION

Preeclampsia (PE) is a very complex and diverse complication of pregnancy. This pregnancy-specific syndrome is specified by extensive disruption in endothelial function of mothers due to circulating factors arising from the placenta. PE is called a disease of theories due to its multifactorial causes. Over the century the concept about PE has been changed from a kidney specific disease to a state of toxaemia (Rana *et al*., 2019). PE is the major cause of maternal morbidity and death around the globe. Currently, no exact treatment option is available for PE other than abrupt delivery of the fetus and placenta together (Khaliq *et al*., 2018). In PE there is alteration in number of metabolic pathways including lipid metabolism, energy metabolism, oxidative stress and insulin resistance (Lopez-Jaramillo *et al*., 2018).

Insulin resistance (IR) is one of the underlying mechanism of PE and is most likely significant from the perspective of preeclampsia risk. IR and hypertension are strongly correlated and behind multiple metabolic abnormalities frequently associated with hypertension, IR is the main diagnostic marker (Ghosh *et al*., 2017). Insulin metabolism, its peripheral action, the availability and impairment of its receptors may play a significant role in vascular disease, leading to hypertensive status in pregnancy (Sáez *et al*., 2019). In pregnancy, insulin resistance acts as an adaptive response to the increasing requirement of the nutrients by the pregnant women and the developing fetus. With the growth of placenta, IR develops early in second trimester and increases with gestational age (Banerjee, 2018). Homeostatic model assessment of insulin resistance (HOMA-IR) is a surrogate marker for IR and have equal ability to predict insulin sensitivity. It is related to fasting levels of glucose and insulin. HOMA-IR is the way of measuring IR in terms of an equation (Vilela *et al*., 2016).

Asprosin, the C-terminal cleavage product of profibrillin, encoded by FBN1 gene, is a novel fasting-induced, glucogenic hormone. Asprosin is encoded by the 2 exons of FBN1. Exon 65 encodes 11 amino acids, whereas exon 66 encodes 129 amino acids. The molecular weight of human plasma asprosin by SDS-PAGE electrophoresis is around 30 kDa. In circulation asprosin is present in nanomolar levels and is secreted by white adipose tissue (Romere *et al*., 2016).

Asprosin crosses blood brain barrier and activates Agouti-related peptide neurons to increase appetite, dietary intake and weight of the body by controlling appetite stimulation in brain. Conversely, a genetic deficiency of asprosin leads to neonatal progeroid syndrome, characterized by low appetite and extreme leanness (Hoffmann *et al*., 2020). In fasting state, asprosin is secreted in circulation and promotes hepatic glucose production. The increase in glucose in turn induces insulin secretion in pancreas. A single injection of asprosin causes a swift rise in blood glucose and insulin in mice, similar to humans, while a reduction in asprosin improves insulin sensitivity and reduces appetite and body weight in mice (Zhang *et al*., 2020).

Many researches have emphasized on the relationship between the circulating level of asprosin and IR and reported that elevated levels of asprosin are seen in

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human with IR-related conditions like type 2 diabetes mellitus and obesity (Yuan *et al*., 2020). However, the role of novel hormone asprosin in preeclampsia is not clear yet.

MATERIALS AND METHODS

Study design and population

The study design was comparative cross sectional and was approved by Committee of Ethical Review, University of Health Sciences, Lahore, Pakistan

Our study comprised 21 diagnosed preeclamptic pregnant mothers and 21 healthy pregnant mothers. The exclusion criteria were as follows; 1) severe anemia 2) hepatic and renal dysfunction 3) T2DM or GDM 4) chronic hypertension 5) chronic inflammatory diseases. The inclusion criteria were 1) singleton pregnancy 2) age between 20-40 years 3) gestational age >36 weeks. Healthy pregnant mothers with blood pressure ≤120/80 mmhg and with absence of proteinuria were included in the control group. The women having increased blood pressure (≥140/90 mmHg) measured at two separate occasions which are at least 6 hours apart and protein of >100mg/dl by urine analysis or >300milligram in a 24-hour urine collection, after 20 weeks of gestation were diagnosed as preeclamptic according to ACOG (2013) and were included in preeclamptic group (Rana *et al*., 2019).

Sample collection

All participants gave their informed consent to participate in the study. Samples from all the participants were taken in operation theatre where healthy and preeclamptic pregnant mothers were on list for elective and emergency caesarean sections. This was done to standardized the protocols of study and to avoid the stress of simple vaginal delivery. Information about whether the women fasted for ≥8 hours before caesarean section was collected. Fasting was mandatory before caesarean section to reduce the risk of aspiration of gastric contents. A total 5ml of blood was drawn under aseptic conditions from the median cubital vein from anterior aspect of forearm of preeclamptic and healthy pregnant women at the time of delivery.

Preparation of serum

The blood was collected in gel containing serum separation tubes. After clotting the blood was centrifuged at 3000 rpm (revolutions per minute) for 10 minutes. The serum was transferred in properly labelled autoclaved eppendroff tubes and was stored at -80 degree Celsius for subsequent biochemical analysis.

Biochemical analysis

Serum asprosin, insulin and glucose levels were measured by using commercially available kits on ELISA analyzer and chemistry analyzer respectively.

HOMA-IR index was measured by using the given formula:

$$\text{Homa -IR} = \frac{\text{Fasting glucose} \times \text{Fasting insulin}}{22.5}$$

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS 21. Normality of the data was checked by Shapiro-Wilks test and was presented as mean ± standard deviation. Independent t-test was used to compare the means of two groups. Pearson correlation test was applied. A p-value ≤ 0.05 was considered as statistically significant.

RESULTS

Demographic and biochemical parameters of mothers from medical record

Of the total 42 mothers in the study, 50% were preeclamptic and 50% were healthy pregnant mothers. The gestational age, uric acid, AST, ALT, urea and creatinine were significantly higher in preeclamptic group as compared to control group. Maternal age and parity were similar in both groups (table 1).

Biochemical estimation

Serum asprosin level in preeclamptic and healthy pregnant mothers

The level of asprosin hormone was significantly increased in preeclamptic group as compared to control group (p=0.000). Mean ± standard deviation is shown in fig. 1.

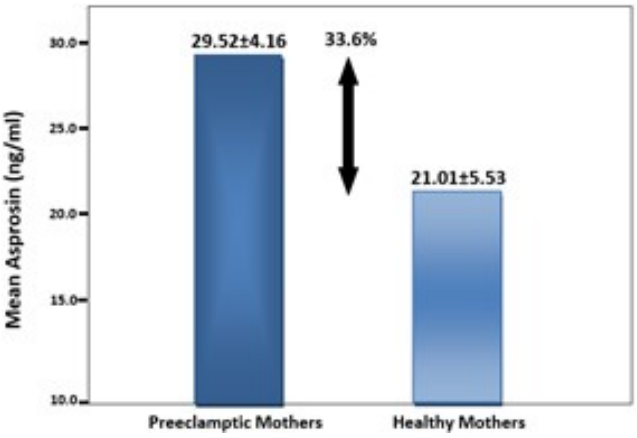

Fig. 1: Serum asprosin level in preeclamptic and healthy pregnant mothers (p=0.000).

Serum insulin level in preeclamptic and healthy pregnant mothers

The level of insulin hormone was significantly increased in preeclamptic group as compared to control group (p=0.003). Mean ± standard deviation is shown in fig. 2.

Table 1: Demographic and biochemical parameters of mothers from medical record.

	Healthy Mothers n=21	Preeclamptic Mothers n=21	*P*-value
Age (years)	26.09±2.58	27.24±3.35	0.223
SBP (mmHg)	118.86±7.90	153.14±7.68[a,b]	0.000
DBP (mmHg)	76.19±6.17	104.57±10.41[a,b]	0.000
Parity	2.52±1.12	2.29±1.01	0.474
GA (weeks)	38.39±0.52	37.53±0.43[a,b]	0.000
Uric Acid (mg/dl)	3.52±0.57	4.95±0.85[a,b]	0.000
ALT (U/L)	21.85±2.54	27.95±3.28[a,b]	0.000
AST (U/L)	26.09±4.08	32.14±3.68[a,b]	0.000
UREA (mg/dl)	23.52±2.82	26.43±3.34[a,b]	0.004
CREATNINE (mg/dl)	0.73±0.10	0.91±0.11[a,b]	0.000

All values are means ± standard deviations, [a]$P<0.05$, , [b]$P<0.01$ vs controls

Table 2: Correlation of serum asprosin with different variables among preeclamptic and healthy pregnant mothers.

	Healthy mothers		Preeclamptic mothers	
	rho(ρ)	p-value	rho(ρ)	p-value
Insulin (µIU/ml)	0.207*	0.369	0.071*	0.759
Glucose (mmo/l)	0.259*	0.256	-0.055	0.814
Homa IR	0.313*	0.167	0.066*	0.776

Correlation is significant at the ≥ 0.01 level.

Serum glucose level in preeclamptic and healthy pregnant mothers

The level of glucose hormone was significantly increased in preeclamptic group as compared to control group (p=0.036). Mean ± standard deviation is shown in fig. 3.

Serum HOMA-IR index in preeclamptic and healthy pregnant mothers

The HOMA-IR index was significantly raised in preeclamptic group as compared to control group (p=0.002). Mean ± standard deviation is shown in fig. 4.

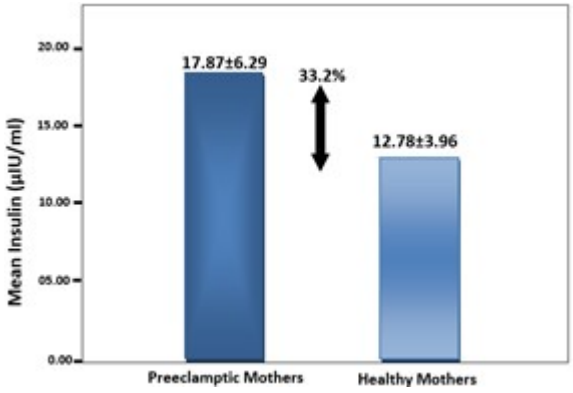

Fig. 2: Serum insulin level in preeclamptic and healthy pregnant mothers (p=0.003).

DISCUSSION

Asprosin, a novel cytokine produced from white adipose tissue is increased abnormally in mammals with insulin resistance. Many researches have emphasized the relationship between the circulating levels of asprosin and IR (Zhang *et al*., 2020).The current study was designed to find out whether asprosin plays a part in the pathogenesis of insulin resistance in preeclampsia or not.

In current study, we observed 33.6% increase in serum asprosin hormone in preeclamptic mothers as compared to healthy pregnant mothers, as specified in fig. 1. Our result was similar to a previous research that observed increased level of serum asprosin in preeclamptic mothers as compared to healthy pregnant mothers (Baykus *et al*., 2019). Asprosin is reported to be elevated in mice and humans with insulin resistance (Yuan *et al*., 2020).

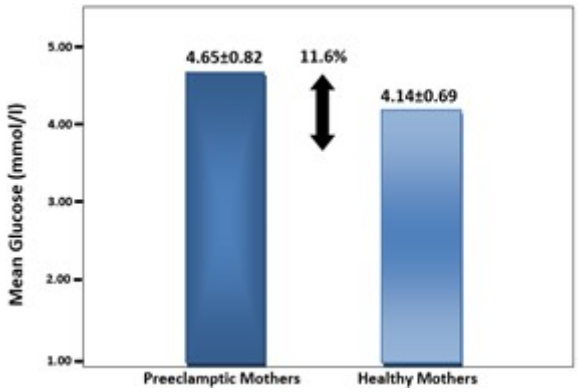

Fig. 3: Serum glucose level in preeclamptic and healthy pregnant mothers (p=0.036).

Metabolic alterations causing insulin resistance and hyperinsulinemia are the prominent factors of preeclampsia (Lopez-Jaramillo *et al*., 2018). We also

observed these factors in our study (figs. 2 & 3). The increased level of serum asprosin in our study is in agreement with an earlier study performed on mice which showed that recombinant asprosin promotes insulin resistance in skeletal muscle cells through stimulation of endoplasmic reticulum stress/inflammation mediated pathways (Jung *et al*., 2019). This could be the possible mechanism of action of asprosin hormone in preeclamptic pregnancies causing insulin resistance. As far as we are aware, asprosin being a novel hormone, is not studied in preeclamptic pregnancies till date.

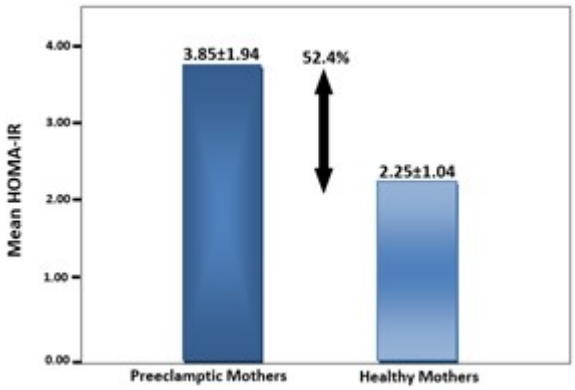

Fig. 4: HOMA-IR index in preeclamptic and healthy pregnant mothers (0.002).

Our study demonstrated that fasting insulin and glucose levels were significantly increased in preeclamptic mothers as compared to healthy pregnant mothers (p=0.003, p=0.036 respectively) (figs. 2 & 3). These findings were in line with Shahzya *et al*. who found similar results in a study on healthy and preeclamptic pregnant mothers undergoing caesarean section (Huda *et al*., 2014). Similar obsevation was made by Ghosh *et al*, who reported significantly elevated fasting glucose and insulin levels in preeclamptic mothers in third trimester (from week 27 to the end of pregnancy) than healthy pregnant women (Ghosh *et al*., 2017). However, Sinha *et al*., reported that mean fasting insulin levels were significantly lower in preeclamptic women than healthy pregnant women and mean fasting glucose levels were not remarkably different in study and healthy groups, in third trimester (Sinha *et al*., 2014). According to our results asprosin, glucose and insulin were increased by 33.6%, 11.6% and 33.2% respectively, in preeclamptic mothers as compared to healthy pregnant mothers. An earlier study indicated a direct role of asprosin in glucose production by hepatocytes (Romere *et al*., 2016). The elevated levels of asprosin enhanced the production of glucose by hepatocytes. Increased glucose production in turn causes increased insulin release from β-cells of pancreas. Due to higher insulin resistance in pregnancy, more insulin was secreted to maintain levels of glucose in normal range (Baykus *et al*., 2019). Perhaps this could be the reason for increased glucose and insulin levels in preeclamptic females in our study.

In current study, insulin resistance was calculated by HOMA-IR. We found a major increase of 52.44% in HOMA-IR index in preeclamptic mothers as compared to healthy pregnant mothers (fig. 4). The findings were in line with multiple studies which reported elevated HOMA-IR index in preeclamptic pregnant women than healthy pregnant women with statistically significant differences (Ghosh *et al*., 2017, Anim-Nyame *et al*., 2015). In contrast to this, multiple researchers found no significant difference in HOMA-IR index between preeclamptic pregnant women and healthy pregnant women (Akdemir *et al*., 2020, Stepan *et al*., 2012, Kun, 2011). Insulin resistance plays a role in pathogenesis of preeclampsia by activation of sympathetic nervous system through stimulating α-1 receptors in muscles and liver (Saxena *et al*., 2018) and this activation causes increased expression of endothelin-1 receptors hence leading to increase blood pressure (Coelho *et al*., 2018). This might be the reason for increased HOMA-IR index in preeclamptic mothers in our research.

Present research found a very weak positive correlation between asprosin and insulin resistance in preeclamptic mothers (ρ=.066) (table 2). Wang *et al*, found positive correlation between asprosin and HOMA-IR index in gestational diabetic pregnancies suggesting circulating asprosin as a predictor of early diagnosis in diabetes (Wang *et al*., 2018). However, their study had not addressed preeclamptic pregnancies. To the best of our knowledge, no reports correlate the effect of asprosin on insulin resistance in preeclampsia pathogenesis until now. This positive correlation between asprosin and insulin resistance is might be due to asprosin induced inflammation, dysfunction and apoptosis of β-cell through toll like receptor 4/ c-Jun- terminal kinase (JNK) mediated signalling leading towards insulin resistance (Lee *et al*., 2019). Toll like receptors are part of innate immune system and JNK pathway controls multiple cellular processes including cell proliferation, embryonic development and apoptosis. Further studies are required for confirming the mechanism of actions of asprosin hormone on insulin resistance in pathogenesis of preeclampsia.

CONCLUSION

These findings support that serum asprosin hormone could be a strong biomarker in the diagnosis of preeclampsia and insulin resistance and possible measures to reduce asprosin and insulin resistance may help in reduction of increased risk of hypertensive disorders during pregnancy. In conclusion we believe that effective and large scale studies are required to clarify the balancing and regulation of asprosin expression in pregnancy and its association with preeclampsia as well as functional studies should be conducted to identify the receptors for asprosin.

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