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Original Article

Maternal Serum Copeptin As A Biomarker For Intrauterine Growth Restriction

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ABSTRACT

In obstetrical world, Intrauterine Growth Restriction (IUGR) occupies second slot as a cause of small for gestation neonates, first being premature birth, both of which result in potential neonatal morbidities and mortalities. IUGR is defined as an estimated fetal weight at one point in time at or below 10th percentile for gestational age. Annually about thirty million babies suffer from IUGR and out of these about 75% are Asians. IUGR has been found to be associated with increased levels of Copeptin. As copeptin is a marker of endogenous stress, so increased copeptin levels can indicate fetal and maternal stress in IUGR. **Objectives:** The objectives of this study were to the compare maternal serum copeptin levels in pregnancies with IUGR and pregnancies with adequate for gestational age fetuses and to establish the significance of copeptin as a biomarker for IUGR. **Methods:** It was a cross-sectional comparative study in which maternal serum copeptin levels were measured and compared in 60 patients divided in two groups, pregnancies with IUGR and normal pregnancies with adequate for gestation age fetuses between 28-35 weeks of gestation. **Results:** Maternal serum copeptin levels were raised in pregnant women with IUGR as compared to that in pregnant women with adequate for gestational age fetuses. Mean ± SD maternal serum copeptin levels were 97.5 ± 6 pg/ml in pregnant women with AGA fetuses and 121 ± 7.8 pg/ml in pregnant women with IUGR. **Conclusions:** Maternal serum copeptin levels are raised in pregnancies with IUGR as compared to pregnancies with adequate for age fetuses which can represent as a possible clinical biomarker for identification of IUGR

INTRODUCTION

In human life cycle, intrauterine period of growth is most important period which has direct influence on birth weight [1]. Birth weight is one of the most significant and easily measurable predictor of morbidity and mortality of infant as well as developmental problems of children, yet this variable is the most ignored one. Infant mortality is believed to be inversely related to birth weight [2]. About 2/3rd of these low birth weight infants are consequence of preterm birth but 1/3rd of these suffer from growth restriction during intrauterine life. The birth of the fetus at or before 28 weeks of gestation is defined as extremely preterm birth [3]. Preterm birth alone is responsible for about 28% of early neonatal deaths. About 45-50% of the preterm births are idiopathic. In 30% cases premature rupture of membranes (PROM) is the culprit while in 15-20% of the incidences of preterm birth, elective premature delivery is opted [4]. According to WHO if the weight of neonate is less than 2500 g it is termed as small for gestational age [5]. Most of the available data on IUGR is collected from the West which cannot be fit on Asian population as it has its own genetic makeup and risk factors. Prevalence of IUGR above 20% is labeled as an alarming situation for public health. The incidence of IUGR in developed countries is about 3% which is far less than in developing countries. The accurate rate of occurrence of IUGR in Pakistan is still vague because of malpractices of deliveries at home and not weighing babies at time of birth, hence making data collection cumbersome and results in underestimation of the problem. In developing countries, prevention of Low Birth Weight (LBW) must be dealt on priority basis because in developing countries IUGR is the major culprit of LBW. In subcontinent 54% of the LBW infants fall under the category of IUGR [6]. About 52% of the

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still birth cases and 10% of perinatal deaths are consequence of IUGR. Moreover, about 72% of idiopathic fetal mortality is shown to be related to IUGR [7]. About 23.8% of the newborns suffer from IUGR. Annually, approximately thirty million babies get affected by IUGR and out of them about 75% are Asians. Unfortunately in Pakistan there is 10-25% incidence of IUGR which is not a healthy sign [8]. In short, these IUGR infants are at an increased risk of metabolic syndrome (comprising Diabetes Mellitus type 2, obesity, dyslipidemias, hypertension and insulin resistance). Long term effects of IUGR on endocrine system of body comprise of polycystic ovarian disease, precocious puberty, short stature etc [9]. The identification of a biomarker will not only be useful for the patients but also for screening of family members and identifying the population at risk and providing preventive treatment at an early stage. Biochemical substance, which involves examination of tissues and body fluids, is currently the most important criteria in quest of a biomarker [10]. Holwerda was the first scientist who described copeptin in 1972. Copeptin (CT-AVP) is also known as COOH – terminal pro arginine vasopressin. It is regarded as a neuroendocrine hormone of hypothalamic hypophysial axis [11]. It is encoded by the same gene that encodes arginine vasopressin. It is located on 20th chromosome position 13 in tandem fashion and reverse order with oxytocin gene.It is synthesized as preprohormone (168 amino acids) in preoptic and paraventricular nuclei of hypothalamus which is broken down by a series of reactions mediated by endopeptidases, mononoxygenases and lyases respectively into prohormone. This segmentation is completed at level of neurohypophysis [12]. Stressful conditions in body stimulate various hormonal, autonomic and behavioral responses which are helpful in short term and long term adaptation to stress. In this regard hormones of Hypothalamic-Pituitary-Adrenal (HPA) axis especially AVP and CRH are of vital importance [13]. The potential mechanism of association between IUGR and copeptin is still debatable. However, importance of copeptin as a potential biomarker of stress is established. Elevated cord copeptin levels depict fetal stress like asphyxia and decreased perfusion of placenta. IUGR has been found to be associated with increased levels of copeptin in a recently published study. Activation of HPA axis by psychological stress has been suggested as one of the potential cause of association between copeptin and IUGR. It is observed that in conditions of perinatal stress e.g. IUGR the vasopressin - copeptin system of neonates as well as mother gets stimulated. In a recent research study it was pointed out that umbilical artery resistance and maternal copeptin are inversely proportional to each other depicting effect of copeptin on function of endothelial cells. The role of AVP as a vasoconstrictor is documented [14]. AVP and VEGF tends to increase resistance of blood vessels and leads to fetal asphyxia, a significant cause of IUGR [15]. Raised copeptin levels are associated with LBW, decreased head circumference, low Appearance, Pulse, Grimace, Activity, and Respiration (APGAR) score, less gestational age and decreased fetal growth. This inverse relation supports the association between copeptin and IUGR [16]. The purpose of current study was to determine maternal serum copeptin levels in pregnant women with AGA fetus and pregnant women with IUGR fetus. Maternal serum copeptin levels in pregnant women with appropriate for gestational age (AGA) fetus and pregnant women with IUGR d fetus were compared. Maternal serum copeptin with age, hemoglobin, history of abortion and gravida score in pregnant women with AGA fetus and pregnant women with IUGR fetus were compared.

METHODS

Thiscross-sectional comparative study was conducted in University of Lahore Teaching hospital, Lahore in collaboration with Obstetrics and Gynecology department, Jinnah hospital, Lahore. A sample size of 30, age range of 20-40 years, in each group was estimated by using Convenient (non-probability) sampling technique and 99% confidence level.The group A comprised of 30 pregnant women (between 28-35 weeks of gestation) with adequate for age uncomplicated normal pregnancy and group B included 30 pregnant women (between 28-35 weeks of gestation) with IUGR pregnancy. The females who had the history of twin pregnancies or multifetal gestation, Oligo/polyhydroamnios, Chronic Hypertension and Diabetes Mellitus were excluded. After selection of patients, written informed consent was taken from them. Personal data including name, age, weight, height, education, employment status were recorded in the questionnaire. Body weight was measured in kilogram by weighing machine available in outpatient department and ward. Height was measured with the help of stadiometer. With the help of weight and height calculation of body mass index (BMI) was done by the following formula. BMI=weight (kg)/ [height (m)].Obstetrical history comprising of gravida score (number of times the woman have become pregnant), parity (no of times the woman have given birth to viable fetus), history of abortion, history of IUGR and duration of pregnancy was taken and recorded.Physical examination of the patients was done to record fundal height and abdominal circumference. An obstetrical ultrasound was done by a trained ultrasonologist to confirm intrauterine growth restriction and adequate for gestational age pregnancy. Umbilical artery Doppler scan

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was evaluated to rule out constitutionally small fetus. Hemoglobin levels (g/dl) were recorded. Blood pressure of the patients was taken to rule out hypertension. A 3 ml blood sample was drawn from anterior cubital vein by using venipuncture method under aseptic conditions. The sample was taken only once as it is a cross sectional study. The blood sample was centrifuged to separate serum. Serum was aliquoted and kept frozen at -20˚C till the estimation of serum copeptin. Test was done by ELISA Technique in the biochemistrylaboratory.For the estimation of copeptin levels, one human copeptin ELISA kit was used (LS Bio Human copeptin ELISA kit LS-F5248-1).The data were analyzed using SPSS (Statistical Package for Social Sciences) version 20. Mean ± SD and median with inter-quartile range was given for quantitative variables i.e., age, gravida score, duration of pregnancy (weeks), hemoglobin and Copeptin level. Frequency and percentage was given for history of abortion and IUGR. Shapiro Wilk test was used to check the distribution of data. For not normally distributed data, non-parametric Mann-Whitney U test, and for normally distributed data independent t-test was used to compare the mean difference in quantitative variables between groups. Chi square and Fisher's exact test was used to determine the association of history of abortion and IUGR within the groups. A p-value of ≤ 0.05 was considered statistically significant.

Groups	History of IUGR		Total
	No (%)	Yes (%)	
Group A	27 (90.0)	3 (10.0)	30 (100)
Group B	26 (86.7)	4 (13.3)	30 (100)
P-Value			>0.999

Table 1: Frequency of History of Intrauterine Growth Restriction in both groups(Fisher's exact test)

RESULTS

The mean age of group A was 27.33 ± 0.65 years and mean age of group B was 28.70 ± 0.62 years. The mean gravida score of group A was 2.6 ± 1.3 and mean gravida score of group B was 3.0 ± 1.1. Mann Whitney U test revealed that there was no statistically significant difference in mean gravida score between both groups. (p-value = 0.166). Out of total 60 pregnant females, 41 (31.7%) had history of abortion. In group A, 5 (16.7%) and in group B, 14 (46.7%) females had history of abortion. Chi square test also revealed that there was significant association of history of abortion with study groups. (p-value= 0.012).Out of total 60 pregnant females, 7 (11.6%) had history of IUGR. In group A, 3 (10.0%) and in group B, 4 (13.3%) females had history of IUGR. The proportion of pregnant females was almost same in both groups. Chi square test also revealed that there was no significant association between history of IUGR and study groups (p-value < 0.999) (Table 1).The mean duration of pregnancy of group A was 30.3 ± 2.2 weeks and mean duration of pregnancy of group B was 31.4 ± 2.0 weeks. The mean Copeptin level of group A was 97.5 ± 6.0 pg/ml and mean Copeptin levelof group B was 121.4 ± 7.8 pg/ml. Shapiro Wilk test showed that the data was normally distributed in both groups; therefore, to compare the mean Copeptin level in both groups independent t-test was performed. It was found that the Copeptin level in group B was statistically significant higher as compared to group A. (p-value < 0.001) (Table 2)(Figure 1).

Groups	Copeptin pg/dl			
	Mean ± SD	Minimum	Maximum	P-Value
Group A	97.5 ± 6.0	87.9	110.2	< 0.001
Group B	121.4 ± 7.8	104.6	134.8	

Table 2: Showing comparison of Copeptin pg/dl level between both groups

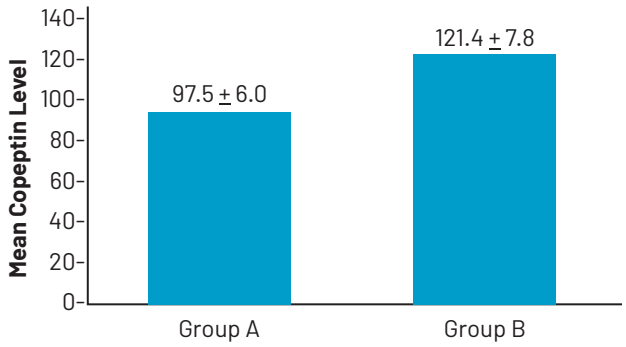

Figure 1: Showing comparison of Copeptin (pg/dl) level between both groups

In Group A, Copeptin level is negatively correlated with age, history of abortion, hemoglobin and gravid score but these correlations were found insignificant. In group B, correlation between Copeptin and age is positive and statistically significant. In group B correlations between copeptin and gravida score, hemoglobin and history of abortion were found to be insignificant(Table 3)(Figure 2-5).

Copeptin pg/dl		Age	History of Abortion	Hemoglobin g/dl	Gravida
Group A	Pearson Correlation Coefficient	-0.022	-0.094	-0.316	-0.190
	P - Value	0.910	0.621	0.089	0.314
Group B	Pearson Correlation Coefficient	0.373*	-0.015	0.076	0.319
	P - Value	0.042	0.939	0.689	0.085

Table 3: Showing correlation of serum copeptin with age, history of abortion, hemoglobin and gravida score
*Correlation is significant at the 0.05 level (2-tailed)

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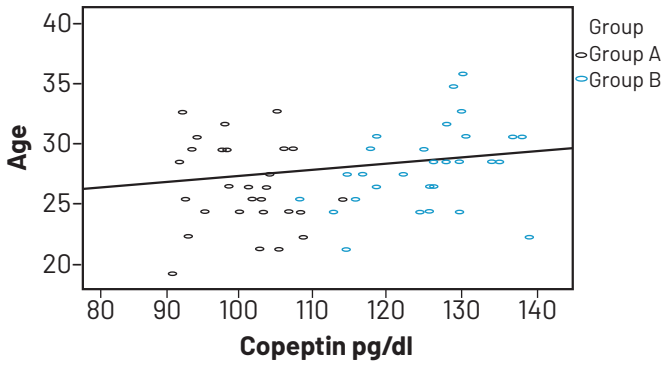

Figure 2: Scatter plot showing correlation between serum copeptin and age in group A and B

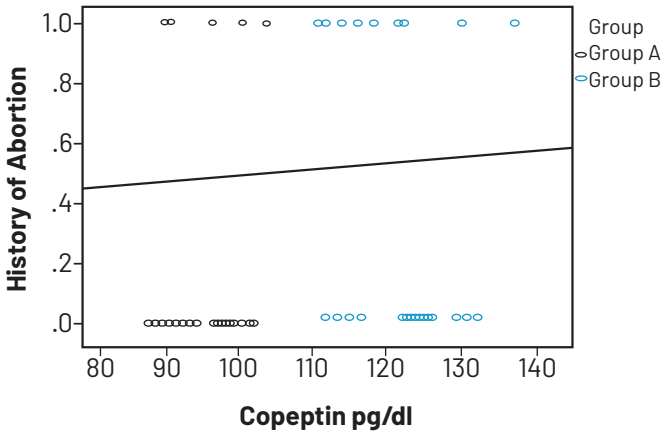

Figure 3: Scatter plot showing correlation between serum copeptin and history of abortion in group A and B

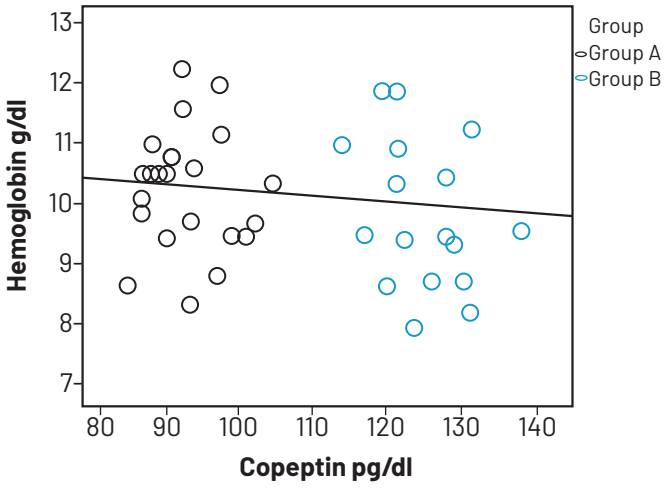

Figure 4: Scatter plot showing correlation between copeptin and hemoglobin in group A and B

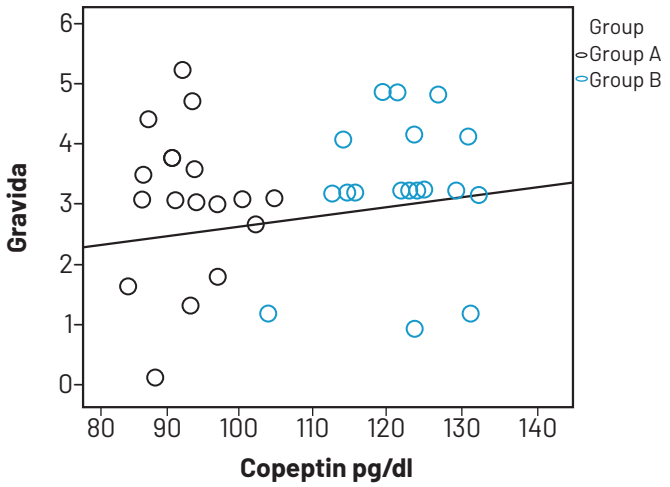

Figure 5: Scatter plot showing correlation between copeptin and gravida score in group A and B

DISCUSSION

In this study we evaluated the effect of maternal age, gravida score, history of previous abortions and IUGR, hemoglobin and serum copeptin levels in pregnant women between the age of 20-40 years with evidence of IUGR by physical examination and ultrasound at 28-35 weeks of gestation with age and duration of pregnancy matched group of pregnant women with evidence of adequate for gestational age fetuses confirmed by both physical examination and ultrasound. In this study the mean age of pregnant women with AGA fetus was 27.33 ± 0.65 years and mean age of pregnant women with IUGR fetus was 28.70 ± 0.62 years. Previous studies pointed out that maternal age < 20 years was a risk factor for LBW [8, 17].The p value of 0.134 in our study depicted that the effect of age on both groups was insignificant. This insignificance can be explained by the fact that none of the women participated in our study was below the age of 20 years. In group A there was positive yet insignificant correlation with copeptin, while in group B there was significant positive correlation between copeptin and age. This finding is different from previous researches carried out by Villela-Torres*et al*. that copeptin doesn't show changes with age [18]. According to our study, the mean gravida score of group A was 2.6 ± 1.3 and mean gravida score of group B was 3.0 ± 1.1(p <0.166). In another study, Manandhar T*et al*. pointed out that as gravida score increases, chances of IUGR decreases [19]. Our study however, has not supported the effect of gravida score on frequency of IUGR. In our study there was no significant correlation found between copeptin and gravid score in both groups. This difference can be evaluated by further studies with emphasis on primigravida women. In the present study, 16.7% of the pregnant women with AGA fetuses and 46.7% of

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pregnant women with IUGR fetuses had history of abortion(p value =0.012. This significant p value is in accordance with the results of research carried out by Hajianfar H, et al. which claimed that women with history of abortion has 2.21% more chances of having IUGR fetus as compared to women without such history [20]. In our study maternal serum copeptin levels were measured to be 97.5 ± 6 pg/ml in pregnant women with AGA fetuses and 121 ± 7.8 pg/ml in pregnant women with IUGR.A recent study pointed out that maternal serum copeptin levels were significantly raised in pregnant women with IUGR as compared to pregnant women with AGA fetuses, (115.76±20.32 pg/ml vs. 98.6±10.19 pg/ml)(p <0.01)and raised maternal serum copeptin levels can be indicative of stress in intrauterine growth restriction. This study also reported significantly raised maternal serum copeptin levels in group with IUGR as compared to group with constitutionally small fetuses (115.76±20.32 pg/ml vs.104.7±9.132 pg/ml)(p<0.05),hence predicting the importance of copeptin to differentiate IUGR from constitutionally small fetuses. However, the difference between maternal serum copeptin levels in group with AGA fetuses and that with constitutionally small fetuses was not significant (98.64±10.19 pg/ml vs. 104.7±9.132 pg/ml) (p>0.904) [16].In another research the significantly raised umbilical cord copeptin levels in pregnancies affected by IUGR as compared to normal pregnancies indicating importance of copeptin as a stress marker [21]. Another study suggested an increased maternal serum copeptin levels in pregnant women with IUGR as compared with pregnant women with AGA fetuses. So our study supports the previous studies that maternal serum copeptin levels are raised in pregnant women with IUGR as compared to pregnant women with adequate for gestational age fetuses which can be helpful in detection of IUGR [22].This difference can be justified by differences in gestational age of the intrauterine growth restricted pregnancies. In this study the gestational age of group with IUGR was 12 weeks and 19 weeks as compared to 28-35 weeks in our study.Our study however showed an increase in maternal serum copeptin in pregnancies with IUGR. So our study supported the association of maternal serum copeptin with IUGR.This study took into account only 60 cases. More authentic and large scale studies should be conducted especially in South Asian pregnant females so that more precise and comprehensive results regarding levels of copeptin in IUGR can be obtained.This study was conducted between 28 to 35 weeks of gestation. Further studies can be conducted during early half of gestation. Raised maternal Serum copeptin levels can prove to be very useful tool in establishment of confirmed diagnosis of IUGR.

CONCLUSION

As very less studies are available on the topic of IUGR and copeptin, this study will be helpful to identify possible association between IUGR and copeptin in Asian population and also raised maternal serum copeptin levels in pregnant women with IUGR can represent as a possible clinical biomarker for identification of intrauterine growth restriction.

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