Serum Copeptin and Neonatal Outcome in Pre-eclampsia

*Sarah Fakhradeen Abdul-Kader, **Ayla Khedher Ghalib

*Azadi Teaching Hospital, Kirkuk, Iraq

**Department of Obstetrics and Gynecology, College of Medicine, Kirkuk University, Kirkuk, Iraq.

Abstract:

Background: Pre-eclampsia (PE) is an idiopathic specific syndrome that affects every organ system during pregnancy.It is a leading cause of both fetal and maternal morbidity and mortality worldwide.
Setting: Antenatal and Obstetric Department in Azadi Teaching Hospital.
Study design: Case-Control study (prospective).
Duration: From April - December 2017.
Patients and methods: The study included 90 pregnant women, who were divided into three groups. 30 normotensive women (control), 30 women with mild pre-eclampsia and last 30 were with severe pre-eclampsia.
Results: Plasma level of Copeptin were 6.87 pmol/l in normotensive pregnant group, 51.70 pmol/l in mild pre-eclamptic group and 54.17 pmol/l in severe pre-eclamptic group (p-value<0.05). Copeptin was significantly elevated in pre-eclamptic subjects compared with controls and in severe pre-eclamptic group compared with mild ones.
Assessing the diagnostic property of Copeptin for PE, (2) still births were recorded in severe PE, while no still births in mild PE and controls.
Copeptin levels in pre-eclamptic patients with adverse neonatal outcomes as low birth weight, admission to NICU were significantly higher than the normotensive group.
Conclusion: This research suggest that increased maternal levels of Copeptin may be involved in the pathogenesis of pre-eclampsia and it may be useful in the assessment of the severity of the disease in the third trimester and this associated with adverse perinatal outcomes.
Key words: Pre-eclampsia, Copeptin, Pregnancy, Neonatal outcome.

Introduction:

Pre-eclampsia is defined as a multisystem disease diagnosed by the characteristic appearance of gestational hypertension of more than 140\90 mmHgandgestational proteinuria which mean urinary protein excretion of more than 300 mg\24 hours, occurring after the 20th week of gestation in a previously normotensive woman and resolving by the sixth postpartum week [(1)]. pre-eclampsia can be detected in less than (5%) of an antenatal population [(2)] and classified as mild and sever pre-eclampsia.

Pre-eclampsia is a multifactorial, polygenic disorder. The risk of pre-eclampsia is positively correlated between close relatives; (20-40%) of daughters and (11-37%) of sisters of women with preeclampsia [(3)].

Copeptin is a 39-amino acid-long, glycosylatedpeptide. It is synthesized mainly in the paraventricular neurons of the hypothalamus and in the supraoptical nucleus. During axonal transport, pre-pro-AVP is proteolytically cleaved into vasopressin, neurophysin II and copeptin[(4).] These

molecules are then stored in secretory granules in the posterior pituitary and released upon osmotic or non-osmotic (hemodynamical; stress-related) stimuli[4].

The concentration of copeptin in the blood circulation ranges from 1- 12 pmol/L in healthy individuals. The levels of copeptine are slightly higher in men than in women and are not influenced by age [5].

Copeptin is the cleavage product of the C-terminal part of pre-provasopressin and has no known function [6].Vasopressin, with a half-life of only 24 minutes,[7] cannot serve as a reliable biomarker [8]. Arginine vasopressin (known as anti-diuretic hormone) is a multifunctional regulatory hormone playing crucial roles in osmotic homeostasis and blood pressure regulation [9], [6].Directly, increased vasopressin may decrease kidney function andincreases blood pressure [6], [5].Indirectly, abnormal endothelial function may lead to decreased vasodilation that alters vasopressin expression [10]. Endothelial dysfunction, which is one of the early stages of atherosclerosis, plays an important role in the pathogenesis of pre-eclampsia [11]. Insulin resistance and low grade systemic inflammation might contribute to the pathogenesis of this endothelial function [11]. Also placenta ischemia secondary to the initial defective placentation and generalized endothelial cell damage and dysfunction creates the pathogenic mechanism underling pre-eclampsia. Thereforewe hypothesized that copeptin may also be predisposing factor in pre-eclampsia. Given that vasopressin may play a role in pre-eclampsia, we measured copeptin, a biomarker of vasopressin, in maternal serum collected blood during pregnancy.In addition, during pregnancy [11]

Pre-eclampsia can cause many complications to the fetus and placenta which included by: Intrauterine fetal death, iatrogenic preterm birth, intrauterine growth restriction and abruption or infarction of placenta [7].

Patients and methods:

This prospective case-control study included 60 pre-eclamptic women and 30 normal pregnant women from the antenatal clinic and labour ward of Department of Obstetrics and Gynecology, carried out in Azadi Teaching Hospital, Kirkuk, during the 1st of April till 31 of December 2017.

The study was carried out in (90) pregnant women who had cards of Antenatal visits to primary health care centers or private clinics. Patients were admitted to the hospital for following up their blood pressure and urine collection for 24 h to estimate the level of albumin during the 2nd and 3rd trimester of pregnancy. They were divided into three groups. The first group included 30 pregnant with normal ongoing pregnancies. The second group consisted of 30 pregnant women with mild pre-eclampsia and the third group included 30 pregnant women with severe pre-eclampsia.

Pre-eclampsia was diagnosed increased blood pressure > 140/90 mmHg that occurred in pregnant women after 20wk of amenorrhea, accompanied by proteinuria ≥ 300 mg/ 24 h.

Severe pre-eclampsia was diagnosed if one or more of the following were present: blood pressure 0f 160/110 mmHg or higher, excretion of 500 mg or more of protein in 24 hour urine sample or urine dipstick showing 3+ or 4+ in a random urine sample on at least

2 occasion measured at least 4 hours apart. All patients in the study were matched for age and gestational age.

The participants were drug native and in 2nd and 3rd trimester. The participants were followed up until they were discharged from hospital.

Inclusion criteria for the study includes the following: Singleton pregnancy, RH +ve mothers,gestational age>20 week and Pregnancies with viable fetus.

Exclusion criteria for the study includes the following: multiple pregnancies, pregnant with urinary tract infection, renal disease, pregnancy induced hypertension, smokers, and alcohol consumers,RH –ve mothers, pregnant with any other chronic disease (DM, thyroid), gestational age<20 week,pregnant women who had any surgical intervention or put on medication for treatment of PE before enrollment were also excluded from the study and pregnant women who had active labour or premature rupture of membranes.

Procedure:

All patients were informed about the purpose of the study and verbal consent was obtained from each participant. Sample collection and storage: 5 ml of venous blood was drawn from patients and put in serum separator tubes (SST) then allowed to clot for 2 hours at room temperature or overnight at 4c ° before centrifugation for 15 minutes at 1000×g. remove serum and assay immediately or aliquot and store samples at -20c° or -80 c°. Avoidrepeated freeze-thaw cycles.

Sample collection and storage: 5 ml of venous blood was drawn from patients and put in serum separator tubes (SST) then allowed to clot for 2 hours at room temperature or overnight at 4c ° before centrifugation for 15 minutes at 1000×g. remove serum and assay immediately or aliquot and store samples at -20c° or -80 c°. Avoid repeated freeze-thaw cycles.

Steps of the procedure:

1- Prepare reagent, samples and standards as instructed.
2- Add 100 µl slandered or sample to each well. Incubate 2 h at 37c°.
3- Remove the liquid of each well, don't wash.
4- Add 100µl Biotin-antibody (1×) to each well. Incubate 1h at 37c°.
5- Aspirate and wash 3 times.
6- Add 100µl HRP- avidin (1×) to each well. Incubate 1 h at 37 c°.
7- Aspirate and wash 5 times.
8- Add 90µl TMB substrate to each well. Incubate 15-30 minutes at 37c°. Protect from light.
9- Add 50µl stop solution to each well. Read at 450 nm within 5 minutes.

Results:

A prospective case-control study included (90) pregnant women for a period of (9) months duration from 1st of April to 31 December 2017.

(30) Pregnant women had mild PE, (30) had severe PE, while the last (30) were normotensive.

Table (1): The mean age for controls was (27.23) year. Range: (18-37), for mild was (30.53) year. Range: (16-42) which was near to the mean age for severe (31.36) year. Range: (18-42), there was no statistically significant differences in between the three groups (P-value= 0.053).

The number of nillparous women was higher in severe pre-eclampsia as shown in table (1) with statistically significant differences (P-value= 0.010). So severe PE associated with 1st pregnancy more than the 2nd and subsequent pregnancies.
Table (2): Our study revealed that early onset of PE were (8), (13) in mild and severe PE respectively, as compared to (22), (17) with late onset diagnosed in mild and severe PE respectively, without statistically significant differences (P-value= 0.176) as sh Table (3): Show that copeptin was higher in pregnant women with severe PE compared with mild PE. There was statistically significant differences between severe PE and elevated Copeptin level (P-value= 0.038) as shown in table (3).
own in table (2).
Table (4): The relation of Copeptin with the onset of PE, those diagnosed at early onset (≤ 32 wk) (21) of them had elevated Copeptin level (>12.5 pmol/l).
As compared to those diagnosed at late onset (>32 wk) only (3) of these had normal Copeptin level with (36) with abnormal Copeptin level. Without statistically significant differences (P-value= 0.192) as shown in table (4), so there was no relationship between Copeptin level and the onset of PE in this study.
Table (5): Show the GA at delivery, (3) of severe PE delivered in between 28-32 wk, (5) in between 33-36 wk, as compared to no deliveries in control and mild groups at these gestational ages. While those delivered at GA > 40 wk, (11) in control, (6) in mild PE with only (3) in severe PE as shown in table (5). There was statistically significant differences (P-value= 0.001). Thus severe PE associated with preterm deliveries either iatrogenic or spontaneous.
Emergency C/S as a mode of delivery was highest in severe PE than in control and mild as shown in table (5). There wasstatistically significant differences (P-value= 0.000) in between severe PE and mode of delivery, that the most severe PE was delivered by emergency C/S.
Table (6):In concerning to birth weight (20) of control delivered with birth weight between (2.5-3.4 kg), only (1) with > 4 kg. While in mild PE (16) delivered between (2.5-3.4 kg), with (1) for each (1.5-2.4 kg) and > 4 kg respectively, as compared to severe PE (7) delivered in between (1.5-2.4 kg), (13) between (2.5-3.4 kg), (5) between (3.5-4 kg), with no babies of > 4 kg among women with severe PE. There was statistically significant differences (P-value= 0.002) between severe PE and low birth weight. Thus pregnant women with severe PE had low birth weight at delivery as shown in table(6).

Table (1): Characteristics of patients included in the study

Maternal characteristics	control	mild	severe	P-value*
Maternal age (Mean) (Range)	 27.23 (18-37)	 30.53 (16-42)	 31.36 (18-42)	 0.053 N.S
Parity Nil parous Multiparous	 9 21	 5 25	 12 18	 0.010 S

*Chi-square test was used*S= Significant*NS= Non-significant.

Table (2): Relationship between onset of diagnosis and severity of PE

Onset of PE	Mild	Severe	Total	P- value
Early(≤ 32wk)	8	13	21	
Late(> 32 wk)	22	17	39	0.176
Total	30	30	60	N.S

*Chi-square test was used*S= Significant*NS= Non-significant

Table (3): Relationship of Copeptin with severity of PE

Level of Copeptin	Mild	Severe	Total	P-value*
<12.5 pmol/l	4	0	4	
>12.5 pmol/l	26	30	56	0.038
Total	30	30	60	S

*Chi-square test was used.*S= Significant

Table (4): Relationship of Copeptin with onset of PE

Level of Copeptin	Early onset ≤32wk	Late onset >32 wk	Total	P-value*
<12.5 pmol/l	0	3	3	
>12.5 pmo/l	21	36	57	0.192
Total	21	39	60	N.S

*Chi-square test was used.*NS= Non-significant

Table (5): Relationship of PE with time and mode of delivery

GA at delivery	Control	Mild	Severe	Total	P-value*
28-32 wk	0	0	3	3	
33-36 wk	0	0	5	5	0.001
37- 40 wk	19	24	19	62	S
>40wk	11	6	3	20	
Total	30	30	30	90	
Mode of delivery					
V.D	8	20	10	38	
Emergency C/S	5	5	17	27	
Elective C/S	17	5	3	25	0.000
Total	30	30	30	90	S

*Chi-square test was used.*S= Significant

Table (6): Relationship between birth weight and PE

Birth Wt	Control	Mild	Severe	Total	P-value*
1-1.4 kg	0	0	5	5	
1.5-2.4 kg	0	1	7	8	
2.5-3.4 kg	20	16	13	49	0.002
3.5-4 kg	9	12	5	26	S
>4 kg	1	1	0	2	
Total	30	30	30	90	

Chi-square test was used.*S= Significant*

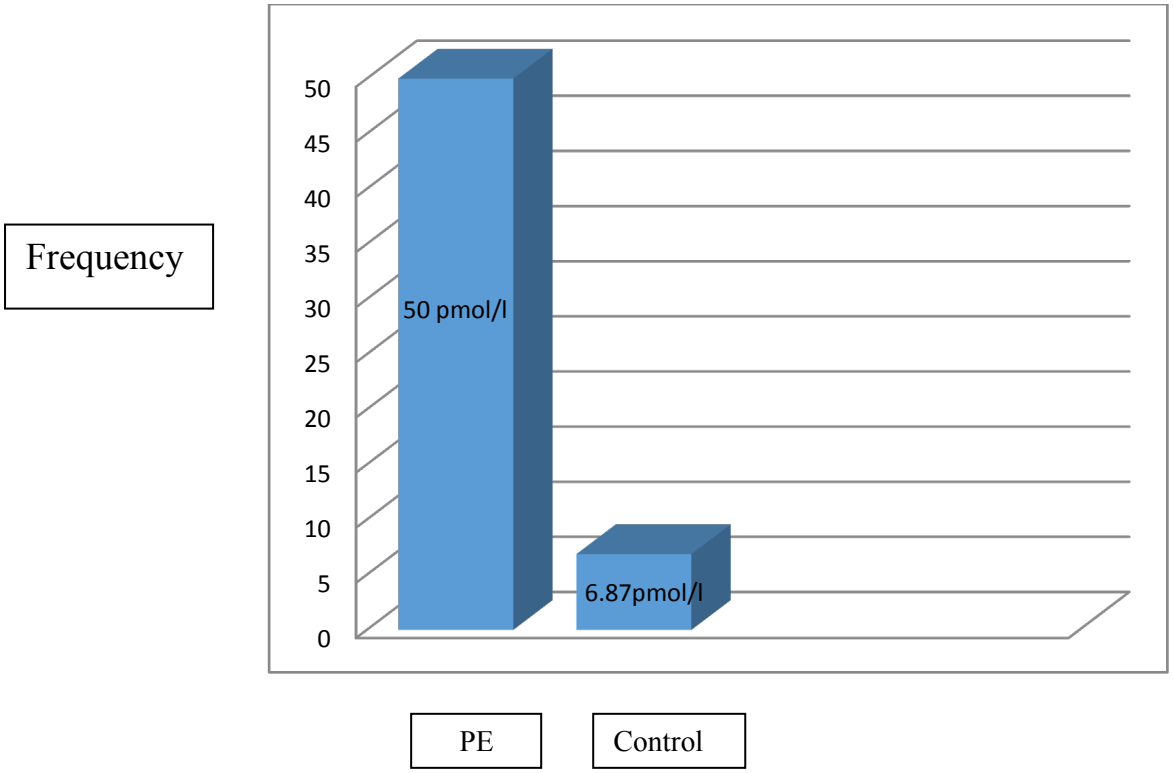

Figure (1): Level of Copeptin between control and PE

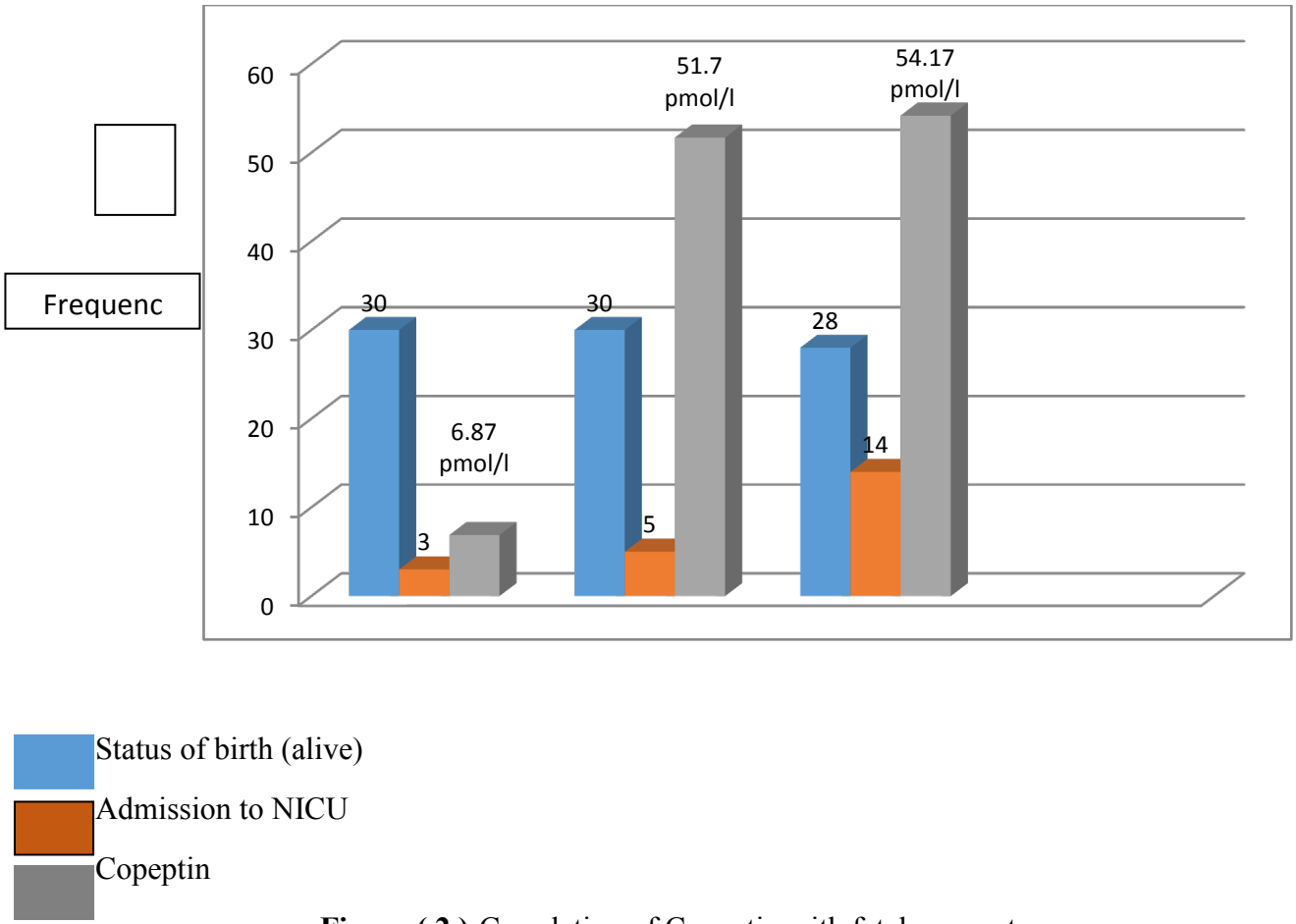

Figure (2): Correlation of Copeptin with fetal parameters

Discussion:

Pre-eclampsia continues to be a major cause of maternal mortality, acute and long-term morbidities,perinatal deaths, preterm birth, and intrauterine growth restriction[11]. In pre-eclampsia, there is elevated BP due to increased level of angiotensin II accompanied by the exaggerated sensitivity of the blood vessels to the increased angiotensin II[12].Furthermore, derangement of endothelial derived vasoactive factors is thought to result in the predominance of substances that are vasoconstrictors over vasodilators in pre-eclampsia.

Mean age of control group of our study was (27.23) years, for mild (30.53) years which was near to the mean age of severe group (31.36) years (p-value= 0.053).No statistically significant differences were observed in patients with pre-eclampsia when compared with their control groups in terms of mean maternal ages at sampling timethat agree with the study which was done by A. Tuten et al [13]. Who found that the level of Copeptin did not correlate with maternal age.

Regarding parity, the highest number of Nulliparity was found in the severe group with statistically significant differences. Our results disagree withA. Tuten et al [13] who foundthat there was no statistical differences were observed in term of percentage of Nulliparity and number of patients with previous PE diagnosis. While Li DK, Wi S et al [14], support that in human, a 30% decreased risk of PE was observed in couples having a second child and subsequent pregnancies who agreed with our study's. Yeung et al [15] evaluated the potential use of copeptin as a predictive model of preeclampsia by examining the association between circulating copeptin and the development of pre-eclampsia.

As our study found that all severe (30) group had elevated copeptin level compared to (26) of mild group. With statistically significantdifferences (P-value= 0.038), agree withZulfikaroglu et al [20] first measured serum levels of copeptin in 32 normotensive, 32 mild pre-eclamptic, and 32 severe pre-eclamptic women during the third trimester of pregnancy. They found that copeptin levels to be significantly higher in pre-eclamptic women when compared with that in normotensive pregnant women. Moreover, women with severe pre-eclampsia had higher serum copeptin levels than those with mild pre-eclamptic. Theconcentration of copeptin in the blood circulation ranges from 1 to 12 pmol/L in healthy individuals[5]. The relationship of copeptin with onset of PE, those with early onset (< 32 wk) all of them (21) had abnormal elevated copeptin level (> 12.5 pmol/l) as compared to late onset (> 32 wk) only (3) of them had normal copeptin level while the other (39) had elevated copeptin level, with no statistically significant differences (P-value= 0.192), these results was agreed with the study done byAkolekar R et al[16], who revealed that the maternal serum copeptin levels are higher in EO-PE and LO-PE patients,but the difference is not statisticallysignificant. Thus, their levels in first trimester are not proven to be effective markers to screen for PE.

Relation of PE with time of delivery in our study, only (3) of severe group delivered at GA between (28-32wk), (5) of severe at GA between (33-36wk), as compared to (0) in both control and mild groups at these GA, while at GA of >40 wk (11), (6), (3) in control, mild and severe, with statistically significantdifferences(P-value= 0.001)

as foundby Steeger et al[17]who support our results by reporting thatthe preeclampsia group exhibited a significantly lower gestational age at delivery (36.1 ± 3.1 vs. 38.8 ± 3.1 weeks, p<0.001), higher andlower blood pressures particularly in the third trimester. These findings are consistent with the known clinical factors associated with preeclampsia: higher rate of preterm delivery, higher rate of twin gestation, and lower birth weight due to vascular causes and earlier delivery, in addition to iatrogenic early termination because of either maternal or fetal complications.

In concerning of the mode of delivery (17) of severe PE delivered by emergency C/S as compared to (5) in both control and mild PE respectively. Withstatistically significant differences (P-value= 0.000) in between severe PE and emergency C/S disagreeing with Gong Y et al. [18] who revealed that most frequent mode of delivery for women suffering from pre-eclampsia is elective C/S.In our study there was higher fetal low birth weight in women with severe PE compared with mild PE and controls with statistically significant differences (P-value= 0.002). Agreeing with a similar observation have been reported by Obed et al[19]support that the birth weights of the newborns were significantly different between the groups and 75% of the patients with pre-eclampsia delivered babies with intrauterine growth retardation (IUGR) and low birth weight, whereas nobabies with IUGR were delivered in the control group.

Regarding status of birth, in this study, there was higher fetal death in women with severe PE compared with no fetal death in both mild PE and controls. In concerning to the admission to NICU (14) of severe group neonates compared to (3) of control group neonates while (5) of mild group neonates admitted to NICU for > 1 day.So that the status of birth, birth weight and the number of neonates admitted to NICU were significantly affected by the level of serum Copeptin and the severity of PE, which was agreed by both Zulfikaroglu et al [20] andSantilan et al[21] revealedthat low birth weight had been associated with increased risk of chronic diseases, mortality and hospitalization. Similarly, 5 min Apgar score < 7 had been shown to have consistent association with neurological disability and low cognitive function in early adulthood.A similar observation had been reported by Kishwara S et al[22]which was attributed to the degree of hypoxia which accompanies PE especially when there is placental abruption which deprives the fetus of oxygen and nourishment and as a consequence, the fetus dies. These poor neonatal outcomes had been attributed to uteroplacental insufficiency and inadequate transport of nutrients. These effects become more pronounced on the fetus as the pregnancy progress and with the severity of PE, due to inability of uterine vasculature to keep up with the increased amount of blood and nutrients necessary for fetal development. Perhaps this explains our observed poorer fetal/neonatal outcomes in infants of severe PE compared with mild PE, therefore the more severe PE the poorer fetal/neonatal outcomes[22].The observed significant negative correlation between maternal serum Copeptin and some fetal/neonatal outcomes suggest that the higher maternal serum level of Copeptin, the lower birth weight, more admission to NICU. This inverse relationship could be due to stress

mediated HPA axis activation (Regulated by AVP or Copeptin) which may precipitate or induce other known humoral, vascular, immune and morphological mechanisms of PE[21]. The limitation of our study is that we didn't measure the parameters of insulin resistance as body mass index because copeptin was associated with measures of insulin resistance (fasting plasma glucose and insulin) and several components ofmetabolic syndrome and dyslipidemia [20].

Conclusions:

It could be concluded from this study that there is elevated maternal copeptin level in pre-eclampsia which increases with severity. Furthermore, copeptin level in the third trimester could predict pre-eclampsia and its elevation is associated with adverse perinatal outcomes. So these women with pre-eclampsia may need increased surveillance during pregnancy.

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