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RESEARCH ARTICLE

Association between placental (pro)renin receptor and small for gestational age in Pre-eclmpsia.

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Abstract

Objective:- Study the association between placental (Pro) renin receptor with the severity of pre-eclampsia and incidence of small for gestational age birth weight.
Method:- 60 pairs of (pre-eclamptic pregnant women and their newborns) admitted to the labour ward with singleton pregnancy, gestational age from 36th to 40th weeks, pregnant women age range between (20-40) years. Exclusion criteria were any abnormalities of placenta, **structural anomalies in the fetus**, multiple pregnancy. The subjects in this study were divided into two main groups: First group contain 30 pairs of pre- eclamptic patients with small for gestational age term newborns. Second group contain 30 pairs of Pre- eclamptic patients with appropriate for gestational age term newborns. Among these Pre-eclamptic women 32 of them were had severe pre-eclampsia and 28 of them were with mild pre-eclampsia.
Result:- The mean of placental (Pro) renin receptor in severe pre-eclampsia was significantly higher (91.4 ng/ml) than the mean of placental (Pro) renin receptor in mild pre-eclampsia (77.54ng/ml) (Pvalue **0.0001**). The results shown that the mean of placental (P)RR in pre-eclamptic women with (SGA) newborns was (88.44 ng/ml) and it was significantly higher (P value **0.002**) than that of the pre-eclamptic women with (AGA) newborns (81.43 ng/ml).
Conclusion:- High placental (P)RR concentration associated with increased severity of pre-eclampsia and high (SGA) birth likelihood.

Introduction:-

SGA neonates define as those with birth weight and/or birth length below the 10th percentile (at least 2 standard deviations (-2 SD) of the recommended gender-specific and gestational age of the population (Lee et al, 2003). This definition is also used in neonatal and obstetrics guidelines . Newborns with SGA weight are 5 times more likely (5 times) to die in the neonatal period and 4.7 times more likely to die in the first year of life (Boguszewski et al, 2011). SGA newborns also associated with increase morbidity rate in neonatal period and adult period (Jancevska et al, 2012). However the etiology of SGA is frequently not well known.

Preeclampsia a pregnancy-specific syndrome characterized by new onset hypertension and proteinuria that affect 5-8% of all pregnancies (Sibai et al, 2005; Roberts et al, 2003). Pre-eclampsia is characterized as severe when (arterial pressure ≥ 160/110mmHg on two occasions, 6 hours apart); proteinuria (≥5 g/24 hours or ≥3+in two urine samples); or any of the following: cerebral/visual disturbances, abdominal pain, abnormal liver function, oliguria, pulmonary edema, thrombocytopenia, or fetal growth restriction (Sibai et al, 2005).

Pre-eclampsia was found to increases the risk of birth complication, but the strength of association varies considerably between studies. Some of this variation may be explained by the fact that most studies do not distinguish between clinical manifestations of pre-eclampsia. Fetal complications in pre-eclampsia are directly related to gestational age and the severity of maternal disease which include increased rates of small for gestational age, preterm delivery, intrauterine growth restriction, placental abruption, and perinatal death (Suppo de Souza et al, 2011).

The Pro renin receptore (P)RR was first identified in mesengial cells in 2002, which is a single transmembrane protein (350 amino acid) that binds renin and its inactive proenzyme form prorenin (Nguyen et al, 2002).

The (P)RR expression has been demonstrated with the highest amount of mRNA found in brain, heart and placenta while lower amount was expressed in liver, pancreas and kidney and other tissue (Nguyen G, 2011). (P)RR involve in regulation of the tissue RAS (Ichihara et al, 2006) and also plays an important role in the function and assembly of vacuolar H+-ATPase (V-ATPase), an ATP dependent proton pump that transports protons across plasma membranes and acidifies intracellular compartments (Kinouchi et al, 2010). Animal studies showed that (P)RR over expression causes slowly progressive nephropathy and hypertension, in addition to activation of the tissue RAS (Kaneshiro et al, 2006; Kaneshiro et al, 2007). The function of the tissue RAS in pregnancy has not been fully elucidated, although previous studies suggest a critical role of the tissue RAS in feto-placental circulation, as well as trophoblast invasion and migration(Irani and Xia, 2008; Hering et al, 2010).

On the basis of these background findings, the present study was conducted to examine the association between placental (P)RR with the severity of pre-eclampsia and birth weight in respect to gestational age in Pre-eclampsia.

Methods:-

Subjects:-

Across section observational study was conducted between October 2015 to the end-June 2016 at Obstetrics and Gynecology department at Al Imamain kadhimain Teaching Medical City. The study protocol was approved by Ethical Committee of College of medicine/ AL-Nahrain University (Baghdad), involved 60 pairs of pre-eclamptic pregnant women with their newborns admitted to the labour ward with singleton pregnancy, gestational age from 36th to 40th weeks which is either calculated by the first day of last menstrual Period (LMP) and/or by Ultasound U/S obtained before 20 weeks of gestation, pregnant women age range between (20-40) years. Exclusion criteria were any abnormalities of placenta, **structural anomalies in the fetus**, multiple pregnancy. B**efore participation all women were given an idea about the study and their written informed consent was taken.** The subjects in this study were divided into two main groups: First group: contain 30 pairs of pre- eclamptic patients with small for gestational age term newborns. Second group: contain 30 pairs of pre- eclamptic patients with appropriate for gestational age term newborns. **Among these** pre-eclamptic women 32 of them were had severe PE and 28 of them were with mild PE.

Sample collection:-

Placental tissue was obtained within 30 minutes after delivery. Incised and weigh 5 gm of placental tissue. Added a given volume of PBS (ph 7.4) and keep at 2-8°C . Homogenization was done use IKA ULTRA- TURAX T25 digital homogenizer at 20,500 rpm for 20 minutes. Then the lysates were centrifuge at 4000 cycles /minutes for 20 minutes and the resultant supernatants were frozen until assay were performed by an ELISA assay (Soluble pro renin receptor assay kit, Takara Bio, Ostu, Japan).

Blood sampling was performed after 1-2 min a prone position and samples were placed in a tube with ethylenediaminetetraacetic acid (EDTA). Then immediately centrifuged at 2,000 rpm for 5 min, stored at -40 C and thawed once prior to measure plasma s(P)RR using ELISA assay (Soluble pro renin receptor assay kit), plasma aldosterone, and plasma renin activity using using ELISA assay(Labor diagnostika Nord LDN) .

Blood pressure was measured to all pregnant women in this study in a prone position on at least two separate occasions with a mercury sphygmomanometer.

In this study all newborns were weighed shortly after birth using neonatal digital scales. Birth weight for gestational age was determined using a global reference for fetal weight and birth weight percentiles (Mikolajczyk et al, 2011). SGA was classified when the birth weight below the 10th percentile for gestational age**.**

Statistical Analysis:-

Data were analyzed by statistical packages of SPSS 18 (statistical packages for social sciences-version 18). All data were presented as a mean ± Sd. Correlation between the variables was performed by spearman correlation coefficient P values were significant if <0.05.

Results:-

From the 32 severe pre-eclamptic women there were 62.5% of them had SGA newborns, while the 28 mild pre-eclamptic women only 35.71% of them had SGA newborns. This mean that there was a significant association between the severity of pre-eclampsia and the incidence of SGA newborns (P value **0.0384),** table **1**.

Table 1. Association of the incidence of SGA and AGA among pre-eclampsia subgroups

Parameters	**Mild PE (n=28) No. (%)**	**Severe PE (n=30) No. (%)**	**P value**
SGA	10 (35.71)	20 (62.5)	**0.0384**
AGA	18 (64.29)	12 (37.5)	
chi square test, * Significant at 0.05 level (two tailed)			

The subjects characteristics association with the incidence of small for gestational age newborns was shown in the table **2.**

Maternal age ,gestational age had no significant difference between pregnant women with small for gestational age newborns and pregnant women with appropriate for gestational age newborns (P value **0.422 , 0.647**) respectively, while the mean BMI for a pregnant women with SGA newborns was (33.08), which significantly lower than that (33.73) for a pregnant women with AGA newborns (P value **0.03).**

The mean systolic and diastolic blood pressure for pre-eclamptic women with SGA newborns were (166.67, 108.33 mmHg) respectively**,** while for pre-eclamptic women with AGA newborns were (155.33, 99.0 mmHg) respectively**,** mean that it were significantly increase in pre-eclamptic women with SGA newborns (P value **0.007, 0.01**) respectively.

The mean birth weight in SGA newborns were (2411.3 gm), which was significantly lower than mean birth weight **(**2797.7gm) in AGA newborns (P value **0.0001**) .

Table 2 : Comparison of maternal age, gestational age, body mass index, blood pressure, and birth weight between small for gestational age (SGA) group and appropriate for gestational age (AGA)group.

Parameters	**SGA (n=30) mean±SD**	**AGA (n=30) mean±SD**	**P value**
Maternal age (yr)	30.83±6.87	29.37±7.19	0.422
Gestational age(wk)	37.03±0.81	37.13±0.98	0.647
BMI (kg/m^2)	33.08±1.3	33.73±0.92	**0.03***
Systolic BP(mmHg)	166.67±18.95	155.33±11.59	**0.007****
Diastolic BP(mmHg)	108.33±11.76	99.00±15.05	**0.01***
Birth weight (gm)	2411.3±233.14	2797.7±231.67	**0.0001*****
SD= standard deviation, Independent t-test, * Significant at 0.05 level (two tailed)			

Table **3.** and Figure **1.** expressed the result of association of placental (P)RR, and plasma s(P)RR with the subgroups of pre-eclampsia. The mean of placental (P)RR in severe pre-eclampsia was significantly higher (91.4 ng/ml) than the mean of placental (P)RR in mild pre-eclampsia (77.54ng/ml) (Pvalue **0.0001**).

Regarding plasma s(P)RR in the cases with severe pre-eclampsia , the results showed that the mean was (45.77 ng/ml) which was significantly high in comparison to(40.29 ng/ml) in the mild pre-eclampsia, (P value **0.005**).

Table 3. Comparison of placental (P)RR, plasma s(P)RR between severe preeclampsia group and mild preeclampsia group.

Parameters	Severe PE (n=32) mean±SD	Mild PE (n=28) mean±SD	P value
Placental (P)RR (ng/ml)	91.4±5.48	77.54±5.4	**0.0001*** **
Plasma s(P)RR (ng/ml)	45.77±7.05	40.29±7.59	**0.005** **
SD= standard deviation, Independent t-test, * Significant at 0.05 level (two tailed)			

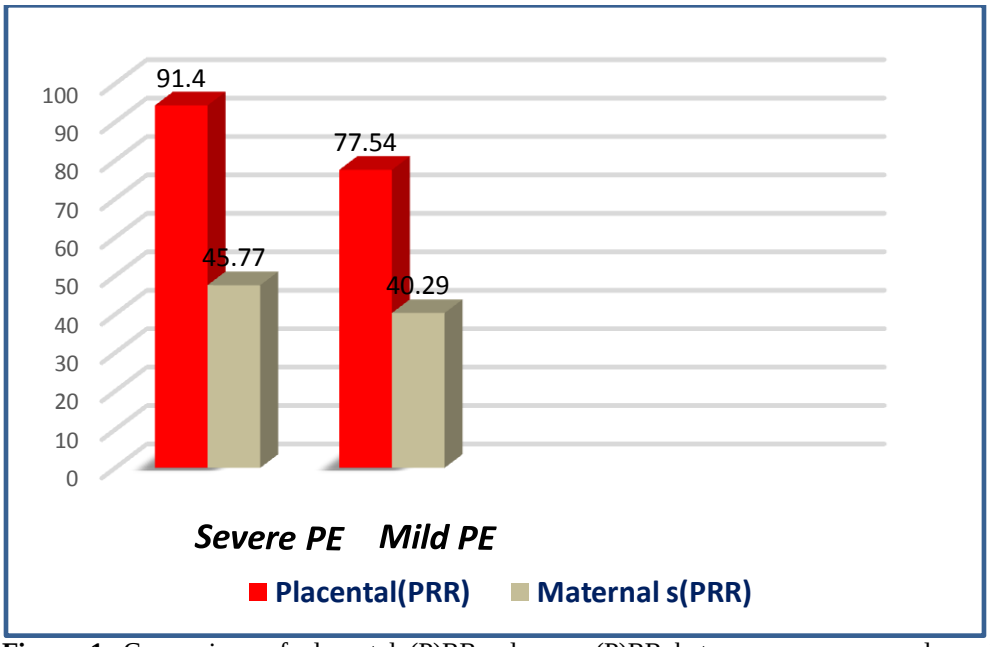

Figure 1. Comparison of placental (P)RR, plasma s(P)RR between severe pre-eclampsia group and mild pre-eclampsia group.

The comparison of placental (P)RR, plasma s(P)RR between pre-eclamptic women with (SGA) newborns and those with (AGA) newborns are shown in the table **4,** and Figure **2.**

The results shown that the mean of placental (P)RR in pre-eclamptic women with (SGA) newborns was (88.44 ng/ml) and it was significantly higher (P value **0.002**) than that of the pre-eclamptic women with (AGA) newborns (81.43 ng/ml).

The results also showed that there was insignificant differences in the plasma s(P)RR between the two groups (P value **0.897**).

Table 4. Comparison of placental (P)RR, plasma s(P)RR between pre-eclamptic women with (SGA) newborns and those with (AGA) newborns**.**

Parameters	Pre-eclampsia with SGA (n=30) mean±SD	Pre-eclampsia with AGA (n=30) mean±SD	P value
Placental PRR (ng/ml)	88.44±7.21	81.43±8.98	**0.002** **
Plasma sPRR (ng/ml)	43.35±5.58	43.08±9.54	0.897
SD= standard deviation, Independent t-test, * Significant at 0.05 level (two tailed)			

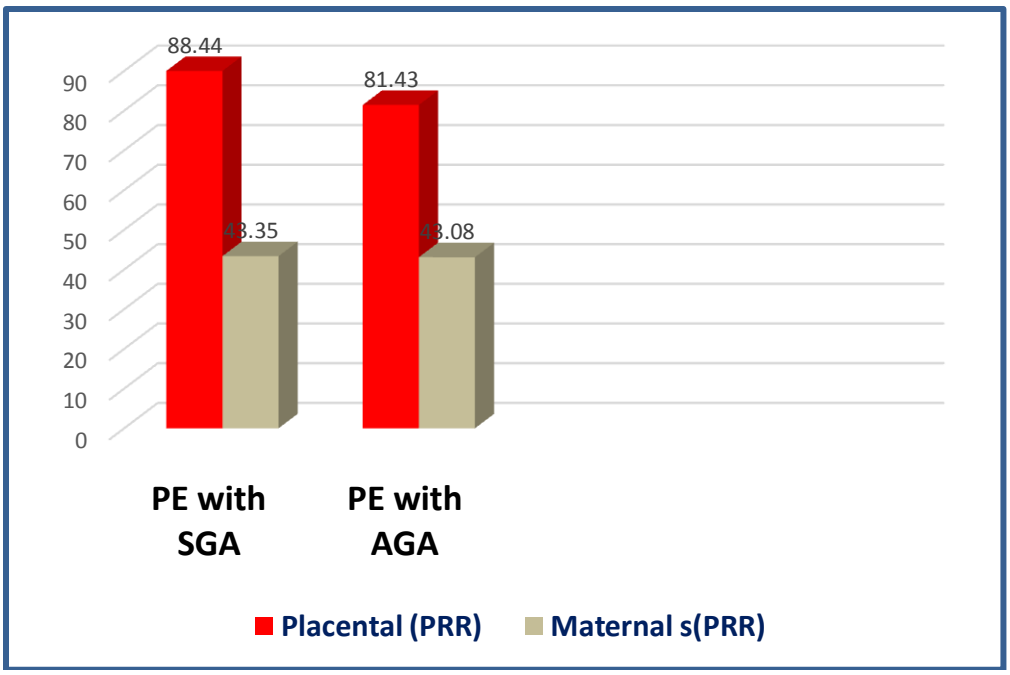

Figure 2. Comparison of placental (P)RR, plasma s(P)RR between pre-eclamptic women with (SGA) newborns and those with (AGA) newborns**.**

In searching for correlation of placental (P)RR) with plasma s(P)RR, table **5,** showed that the plasma s(P)RR, had a significant positive correlation with placental (P)RR (P value **0.026**).

Table 5. Correlation of placental (P)RR with maternal s(P)RR within all patients

Parameters	**Plasma s(P)RR (ng/ml)**	
	r	**p**
Placental (P)RR (ng/ml)	0.288	**0.026***
*** Correlation is significant at the 0.05 level (2-tailed).**		

The current study was dealt with comparison of correlation of placental (P)RR and plasma s(P)RR with birth weight, blood pressure , urin protein creatinine ratio , aldosterone , and plasma renin activity of included Pre-eclamptic women as in table **6**. Birth weight had a significant negative correlations (P value **0.004**) with placental (P)RR while no significant correlations with maternal s(P)RR in which (P value **0.178**).

It had been shown that systolic blood pressure had a significant positive correlation with placental (P)RR (P value **0.0001**), and had no significant correlation with maternal s(P)RR in which (P value **0.053**), while diastolic blood pressure had no significant correlation with both placental (P)RR and maternal s(P)RR (P value 0.060, 0.416) respectively.

In our study the urine protein creatinine ratio had a significant positive correlation with maternal s(P)RR P value was **0.009**, while no significant correlation with placental (P)RRP value was **0.108**. There was no significant correlation between plasma aldosterone and placental (P)RR neither with plasma s(P)RR (P value **0.066**, **0.191**) respectively. Also plasma renin activity had no significant correlation with both placental (P)RR and plasma s(P)RR in which (P value **0.775**, **0.384)** respectively.

Table 6. Comparison the correlation of placental (P)RR and plasma s(P)RR with birth weight, blood pressure, urine protein creatinin ratio, aldosterone and plasma renin activity

Parameters	Placental (PRR)		Plasma s(PRR)	
	r	p	r	p
Birth weight (gm)	-0.368	**0.004****	-0.176	0.178
Systolic BP (mmHg)	0.627	**0.0001*****	0.251	0.053
diastolic BP (mmHg)	0.244	0.060	0.107	0.416
urine protein creatinine ratio PCR	0.209	0.108	0.332	**0.009****
Aldosterone (ng/dl)	-0.239	0.066	-0.171	0.191
Renin (ng/ml.hr)	0.038	0.775	-0.114	0.384
*** Correlation is significant at the 0.05 level (2-tailed).**				

The diagnostic accuracy of placental (P)RR, for detection of small for gestational age newborns was shown in figure **3**. The P value was (0.002), as it had the highest (AUC) 0.736 and the best cut off value was 81.45ng/ml , the specificity was 56.7%, and the sensitivity was 80%.

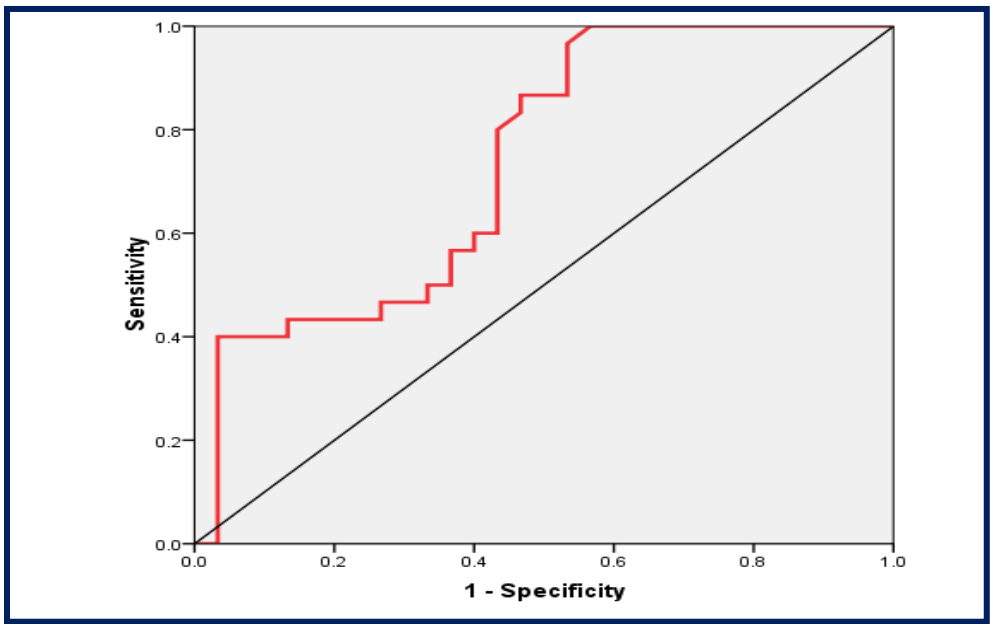

Figure 3: ROC curve for placental (P)RR, and their validity in detecting in small for gestational age.

Discussion:-

This study dealt with association of placental (P)RR, and plasma s(P)RR with birth weight and severity of pre-eclampsia. The mean of placental (P)RR for all pre-eclamptic patients was averaged 84.93±8.82 ng/ml and the mean plasma s(P)RR was averaged 43.21±7.75 ng/ml. These results were higher than a results of a previous cross sectional study involved (15) normotensive pregnant women which demonstrated that the mean of placental (P)RR was 55.9±15.0 ng/ml and the mean plasma s(PRR) concentrations was 24.9±3.7 ng/ml (Nartita et al, 2016). This high placental expression of (P)RR in pre-eclampsia give us a suggestion that placental RAS activation is enhanced in pre-eclampsia.

The current study was the first study to compare the placental (P)RR and plasma s(P)RR between the subgroups of pre-eclampsia. This study stated that there was a significant increase in mean of placental (P)RR and plasma s(P)RR concentration in severe pre-eclampsia when compare with mild form. These results suggest that placental (P)RR, and plasma s(P)RR, involving in pathogenesis of pre-eclampsia. This may be due to effect of oxidative stress in pre-eclamptic placental tissue which heightened by placental RAS, as the ROS superoxide anion (O_2^-) has been involved in Ang II-mediated hypertension, also there was an experimental data established that in pregnancy the Ang II-mediated hypertension, in a part, may be due to the effects of oxidative state on the vascular-endothelial tissue (Mistry et al, 2013; Shah D.M. 2005).

There was a significant increase in mean of placental (P)RR concentration in a pregnant women with small for gestational age newborns in comparison with pregnant women with appropriate for gestational age newborns. This agree with Mistry et al, (2013) who stated that the increased activity of placental RAS, especially evident in the syncytiotrophoblast, may be the contribution to both pre-eclampsia itself and the intra uterine growth restriction. Kurlak et al, (2016) also suggested that the placental RAS responsible for changes in tissue oxygenation and thus affect placental development and function which necessary for fetal growth.

This study also revealed insignificant difference in the mean of plasma s(P)RR between pregnant women with small for gestational age newborns in comparison with pregnant women with appropriate for gestational age newborns. This observation was agree with Watanabe et al, (2013) who found no significant association between maternal plasma concentration of s(P)RR and the incidence of SGA.

We also found that there was a significant positive correlation between plasma s(P)RR and placental (P)RR expression. This observation disagree with previous study Nartita et al, (2016) who observed no correlation between placental (P)RR expression and plasma s(P)RR. However, we can speculate that placental (P)RR may be a major sources of plasma s(P)RR which is cleaved and regulated by furin-like factors then secreted into the maternal blood circulation. Therefore, it is likely that plasma s(P)RR give us a view about the placental (P)RR expression.

In this study, systolic blood pressure was positively correlated with placental (P)RR levels, but not with maternal plasma s(P)RR levels, while diastolic blood pressure was not correlate with each of them. This in accordance with Nartita et al, (2016) who stated that systolic blood pressure was positively correlated with placental (P)RR concentration, but not with plasma s(P)RR levels in pre-eclamptic patients. The correlation between placental (P)RR level and systolic blood pressure in pre-eclamptic patients suggests that placental (P)RR is involved in the regulation of blood pressure through the activated tissue RAS.

On other hand plasma s(P)RR levels had a significant positive correlation with spot urine PCR, while placental (P)RR had no significant correlation with any of them. These results was in accordance with Morimoto et al, (2014);Nartita et al, (2016) who stated that s(P)RR level was associated with decreased GFR independent of blood pressure. Watanabe et al, (2012) was also stated that high s(P)RR levels at delivery may be associated with proteinuria in pre-eclampsia. However its not clear whether plasma s(P)RR levels was increase in pre-eclampsia due to increase tissue (P)RR cleavage, or due to decrease renal excrection.

In this study there was no significant correlation between placental (P)RR, plasma s(P)RR levels with plasma renin activity and aldosterone . This observation was agree with Morimoto et al, (2014); Nguyen et al, (2014) who stated that there was no association between s(P)RR with plasma renin activity and aldosterone. While disagree with Shefe JHea (2006) who established that (P)RR expression was downregulated by renin.

One of limitation in this study was the small sample size therefore the need for further studies are suggested on a larger sample of pre-eclamptic women in each subgroup, and compare them with control normal pregnant women groups. Measure changes in plasma s(P)RR in several variable periods of gestation is recomended, and follow up its association with intra uterine fetal growth. We also need to follow up the changes in maternal s(P)RR after delivery, to study the effect of delivery of placenta on its concentration.

In conclusion the placental (P)RR was significantly high in a pregnant women with small for gestational age newborns in comparison with pregnant women with appropriate for gestational age newborns , and there was a significant negative correlation with birth weight.

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