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The Utility of Serum Total and Placental Alkaline Phosphatase Activity as Predictive Markers for Preeclampsia among Sudanese Pregnant Women

Yusra M Abdallah[1], Ghada A Elfadil[1]. Maazza Omer Adam[1], Abdelgadir Elmugadam[1], Gad Allah Modawe[2*]

1. of Medical Laboratory Science, Sudan University of Science and Technology, Khartoum, Sudan
2. Department of Biochemistry, Faculty of Medicine and Health Sciences, Omdurman, Sudan

Abstract

Background: Serum total alkaline phosphatase (TALP) and placenta alkaline phosphatase (PALP) measurement can afford a simple, reliable and economical adjunctive laboratory parameter in hypertensive disorders of pregnancy.

Objective: To determine the utility of serum TALP and PALP as predictive markers for preeclampsia among Sudanese pregnant females.

Materials and Methods: Cross-sectional comparative hospital-based study conducted in Khartoum state atOmdurman Maternity Hospital from March to June 2022. Hundred Sudanese pregnant women age ranged between (20 to 45 years), 50 pregnant women with preeclampsia and 50 normal pregnant women as control group. The serum TALP, PALP were measured using full automated analyzer (response® 910), while serum urea, uric acid by enzymatic method and creatinine by chemical method. The data obtained was analyzed by using SPSS version (26).

Results: Preeclamptic women showed significant increase in means of serum uric acid (7.4± 2.3 mg/dL VS 3.6± 1.0 mg/dL), urea (33.8± 4.0 mg/dL Vs 17.5 ± 6.2 mg/dL), PALP (151.5± 76.1IU/L VS 111.1±65.4 IU/L, P-value < 0.05) and the ratio of PALP/TALP (0.66 ±0.2 IU/L VS 0.52±0.1 IU/L, P-value< 0.05) compared to normal pregnant women. The Receiver operating characteristics curve (ROC) showed optimum cut-off value of PALP >133 IU/L and ratio >0.589 as predictor of preeclampsia among Sudanese pregnant women.

Conclusion: Sudanese women with preeclampsia had an elevated serum uric acid, urea, PALP and ratio of PALP/TALP. Moreover, PALP and ratio of PALP/TALP can be used as predictive marker of preeclampsia in pregnancy.

Key words: Preeclampsia, Total ALP, PALP, urea, uric acid

Corresponding author: Gad Allah Modawe gadobio77@oiu.edu.sd.

Introduction

Preeclampsia is a complex and serious multi-system disorder of human pregnancy with a worldwide incidence of 5–7% and contributes significantly to maternal and perinatal morbidity and mortality (1). It is characterized by hypertension, proteinuria, and a generalized systemic vasoconstriction arising from circulatory disturbances secondary to a generalized endothelial dysfunction caused by inflammation (2). It is associated with defective endovascular trophoblastic invasion and insufficient remodeling of the uterine spiral arteries (3,4) Altered renal function is an essential component of the pathophysiological process in pre-eclampsia and close monitoring of renal function is important to ensure a timely delivery before serious renal damage occurs.

Several Alkaline phosphatase (ALP) isoenzymes are elaborated in human tissues including placental isoform and as independent genetic loci (5,6). The existence of four human isoenzymes, each encoded by independent genetic loci, has been well documented and includes an intestinal isoenzyme, localized in the brush border of the mucus membrane. A second isoenzyme, derived from placental syncytiotrophoblasts, appears in the second trimester. Placental like ALP (tissue nonspecific), includes enzymes originating from bone, liver, lung, and leukocytes (1).The primary source of human placental Alkaline Phosphatase (PLAP) is the syncytiotrophoblastic plasma membranes, during the second & third trimesters of pregnancy. As gestation progresses, the concentration of PLAP rises till term and this can be caused by the detachment of ALP from the membrane into the maternal circulation (7,8). In normal pregnant women it rises to a level 2-3 times higher than that of non-pregnant women and do possess a long half-life (seven days postpartum) (9). The placental-type human ALP, which often occurs in human non-trophoblastic tumors, has been considered a marker for malignant transformation. Yet, the appearance of this heat-stable enzyme is not universal for human tumors, many of which may produce heat-labile ALP enzymes (10). The objective of present study was to determine the utility of serum TALP and PALP as predictive markers for preeclampsia among Sudanese pregnant females**.**

Materials and Methods

Study population: The study was a hospital-based cross sectional comparative study; carried out after obtaining an ethical clearance from Sudan university of Science and Technology ethical committee and informed consent from each study subject. The study was carried out in the Omdurman Maternity Hospital from March to June 2022. 100 pregnant women (50 hypertensive women and 50 normotensive women) were recruited into the study. Inclusion criteria for preeclamptic: pregnant women with a blood pressure of ≥ 140/90 mmHg, which included patients in all the category of HDP according to the National High Blood Pressure Education Program classification (11).

Exclusion and Inclusion criteria: Inclusion criteria for comparative group: 50 healthy pregnant women with no history of pregnancy related complications, diabetes mellitus or any other chronic medical illness. The age of the study group was between 18 and 35 years and their gestational ages were between 36 and 40 weeks (peak PLAP rise is between 34 and 40 week of gestation). Pregnant women with diabetes mellitus, jaundice, chronic liver disorders, anemia and other pregnancy associated disorder like gestational diabetes, complicated pregnancies (vaginal bleeding after 28-week, fetal distress and congenital abnormalities) were excluded.

Blood sample and data collection: Five ml of blood sample (from both normal and preeclamptic pregnant women) was collected in a

plain vacutainer. The individual samples were centrifuged at 1200 rpm for 10 min and the separated serum was used for the analysis of total alkaline phosphatase (TALP), placental alkaline phosphatase - (PLAP), creatinine and uric acid. The biochemical assays were carried out using procedures approved by the IFCC. The analyses were essentially carried out on the same day within four hours so as to minimize the inactivation of ALP by denaturation. Alkaline phosphatase (12) and uric acid (13) in serum was estimated based on a spectrophotometric method by a fully automated random access chemistry analyzer. The initial values obtained without heat inactivation pertained to the serum total ALP activity. Serum PLAP (heat stable fraction of ALP) was measured by the thermal inactivation method (14). 0.5 ml of sera samples were added into small thin-walled glass tubes placed in thermostatically controlled water bath stabilized at 65^0C. The water level was at least 3 cm above the samples. Exactly following 30 minutes, the serum tubes were rapidly removed and placed in an ice bath for 3 min before returning it to room temperature. The ALP activities of the processed samples were determined similar to that of total ALP and this represented the heat stable fraction of ALP (PLAP).

Statistical analysis: The data was analyzed using the SPSS software, version 26. The values were expressed as mean and standard deviation. The independent Students t-test was performed to compare the mean values in hypertensive and normotensive pregnant women. Pearson's correlation analysis was performed to determine the association between various test parameters. A p-value of < 0.05 was considered to be significant. Receiver operating curves (ROCs) were drawn to elicit the optimum sensitivity, specificity and cut-off values.

Results

One hundred Sudanese pregnant women were included in this study. 50% (n=50) with hypertension disorders of pregnancy and 50% (n=50) were normotensive pregnant women. Their gestation age between 34 and 40 weeks of gestation. Study revealed that there was significant increase in mean of serum PALP, uric acid levels and PALP/TALP ratio in pregnant women with hypertension disorder of pregnancy when compared with counterparts. However; there were insignificant difference in the level of serum creatinine and TALP activity among women with hypertension disorder of pregnancy when compared with normotensive pregnant women (Table1).

In Spearman correlation study revealed that PALP/TALP ratio correlated positively with serum creatinine (Table 2). The Receiver operating characteristic curve (ROC) of PLAP/ALP ratio showed an optimum cut-off at 0.589 with 66% sensitivity and 64% specificity, with a significant area under the curve (AUC=0.695). The PLAP showed an optimum cut-off at 133 with 56% sensitivity and 72% specificity, with a significant area under the curve (AUC = 0.659) (Figure 1).

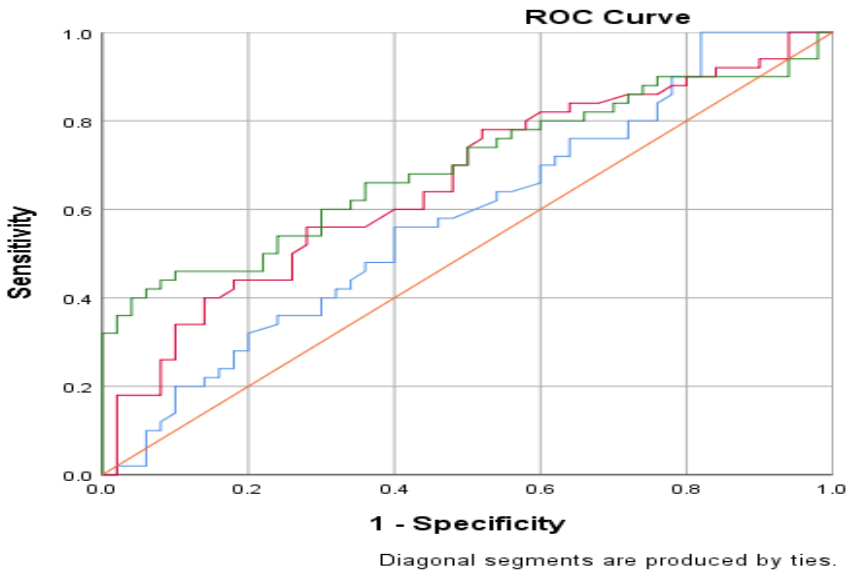
Table 1: Comparison of age, SBP, DBP, urea, creatinine, total and placental alkaline phosphatase in preeclamptic and normal pregnant women.

Parameters	preeclamptic (n =50)	normal pregnant (n= 50)	P- value
Age (year)	27.6 ±7.6	27.6 ±5.2	0.987
SBP mmHg	163.7 ±27.9	108.3 ±10.4	0.000
DSP mmHg	104.2 ±17.8	74.8 ±7.7	0.000
Urea (mg/dL)	33.8 ±4.0	17.5 ±6.2	0.008*
Creatinine(mg/dL)	1.5 ±0.3	0.6 ± 0.1	0.076
Uric acid (mg/dL)	7.4 ±2.3	3.6 ±1.0	0.000*
TALP IU/L	221.8 ± 76.4	204.06 ± 93.2	0.300
PALP (IU/L)	151.5 ± 76.1	111.1 ± 65.4	0.005 *
PALP/TALP ratio	0.66 ± 0.2	0.52 ± 0.1	0.000*

Independent sample t-test was used to compare between means.
P-value considered significant at 0.05.

Table 2: Spearman correlation between age blood pressure urea and creatinine with TALP, PALP and ratio among women with hypertension disorder of pregnancy.

		Age	SBP	DBP	Urea	creatinine	UA
ALP	r	-0.131	0.029	0.019	0.119	0.073	0.231
	P	0.363	0.840	0.898	0.426	0.623	0.122
PALP	r	-0.108	0.039	0.048	0.139	0.220	0.061
	P	0.456	0.788	0.742	0.120	0.137	0.686
PALP/ALP ratio	r	-0.096	0.042	0.087	0.218	0.330	0.216
	P	0.505	0.773	0.550	0.141	0.024*	0.184



Variables	95%: Confidence Interval	AUC	P- value	% Sensitivity	% Specificity	Cut-off
ALP IU/L	0.470 - 0.694	0.582	0.159	56	60	212.0
PALP IU/L	0.552- 0.766	0.659	0.006	56	72	133.0
Ratio PALP/ALP	0.591-0.800	0.695	0.001	66	64	0.589

Figure 1: Receiver operating characteristics curve of total, placental ALP and ratio.

Discussion

The present study showed significant increase of serum PALP in preeclamptic pregnant women when compared to normal pregnant group. This finding agreed with study done by Mishra and his colleagues which found that about 2-fold increased ALP in hypertensive pregnant indicative of placental dysfunction and may be a warning to impending fetal health (***15).*** Moreover; study by Hutchinson and his group found that the elevated levels of serum PALP in hypertensive pregnant women may be attributed to placental dysfunction, which results in increased serum levels of these enzymes. Shedding of syncytiotrophoblast into the maternal circulation is a normal part of pregnancy, but is increased during pre-eclampsia. In pre-eclampsia, this process of syncytio-trophoblast renewal is overactive and complicated by necrosis and apo necrosis of the syncytio-trophoblast particles (***16).*** Also, study showed significant increase in ratio of serum PALP/ALP in case compared to control group. This finding in agreement with study done by Rajagambeeram and his colleagues which found that the ratio of PLAP/ALP was significantly higher in hypertensive disorders of pregnancy with p-value of <0.001.(***17).*** The other aspect in this study is raised serum uric acid level in hypertensive pregnant women to normotensive

pregnant group. Bainbridge and Roberts suggested that hyperuricemia in hypertensive pregnant women is multifactorial (18). In hypertensive pregnant women, elevated levels of uric acid are not only attributed to decreased renal excretion but also to increased oxidative stress resulting from placental ischemia and increased activity of xanthine oxidase enzyme (19). Uric acid is a potent inhibitor of endothelial function, found to induce systemic and glomerular hypertension in animals (20). Also, increased tubular reabsorption and decreased tubular secretion of uric acid in tubules or diminished renal blood flow, similar to the physiologic response to hypovolemia may be the cause for hyperuricemia. Serum creatinine is widely used as an indicator for GFR. Our study revealed that there was a statistically insignificant difference in the mean values of serum creatinine level in hypertensive pregnant women to normotensive pregnant group; these was consistent with the previous study (21). Moreover; study pointed that PLAP/ALP ratio with an optimum cut-off at 0.589 and PLAP with an optimum cut-off at 133 IU/L have diagnostic value in preeclampsia. Rajagambeeram and his group showed a better and practical approach towards the assessment of hypertensive disorders of pregnancy would be to utilize heat stable ALP isoenzyme (PALP) and PALP/ALP ratio as an adjuvant marker in the armamentarium of biochemical tests, especially since the same is simple to assay and reliable as well as economical and sensitive (17).However, the future outcome of preeclamptic pregnant women, which could have provided more information regarding both maternal and fetal outcome could be the scope of future studies.

Our finding considered first study among preeclamptic Sudanese women who assess the cut-off value of PALP and TALP/PALP ratio. However, the limitation of this study is that the outcome of preeclampsia and it is impact on both maternal and fetus was not observed.

Conclusion

The study concluded that Sudanese women with preeclampsia had elevated serum uric acid, PALP and ratio of PALP/TALP. Moreover, PALP and ratio of PALP/TALP can be used as predictive markers of preeclampsia in pregnancy.

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