RESEARCH ARTICLE

IDENTIFICATION OF PREDICTIVE MARKER OF PRE-RENAL DAMAGE IN PREGNANT WOMEN WITH PREECLAMPSIA AND WOMEN AT HIGH RISK – A PROSPECTIVE STUDY CONDUCTED IN RIYADH, SAUDI ARABIA

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ABSTRACT

Background: Preeclampsia is characterized by development of high blood pressure and proteinuria. It affects 5–8% of all pregnancies and is a major contributor to maternal and fetal morbidity and mortality. There is no single test that fulfils all the criteria for a good predictor of preeclampsia and associated renal damage.

Aims & Objectives: To evaluate the role of serum and urine biochemical parameters as early predictors of preeclampsia. To investigate the role of BUN: Creatinine ratio in diagnosing preeclampsia and evaluating prognosis of the disease.

Material and Methods: In the present prospective study, one hundred and twenty pregnant women divided into three groups: normotensive (control), women at high risk and with preeclampsia were included. Analyses of different biochemical parameters including BUN: Creatinine were carried out.

Results: There was significant difference in the mean value of serum uric acid, blood urea nitrogen, creatinine, urinary protein and BUN: Creatinine ratio in preeclampsia group compared to control group ($p < 0.001$). There was significant difference ($p < 0.05$) in serum uric acid between control and preeclampsia group. However, there was no significant change in haematocrit, serum creatinine and urine protein between control and high risk group.

Conclusion: BUN: Creatinine ratio in pregnant women with preeclampsia and also in high risk group was significantly increased ($t = 15.55$, $p < 0.001$ and $t = 8.66$, $p < 0.001$ respectively) in comparison to the control group. This index could be useful in evaluating the severity of preeclampsia and could be used as a predictor in prognosis of preeclampsia and subsequent early renal disease.

Key-Words: Blood Urea Nitrogen: Creatinine Ratio (BUN:Cr); Maternal Mortality; Preeclampsia; Renal Dysfunction

Introduction

Preeclampsia is a multisystem and multifactorial disease that affects both mother and the fetus by vascular dysfunction and by intrauterine growth restriction.[1] It is characterized by development of high blood pressure (hypertension) and proteinuria after 20 weeks of gestation and affects about 5-8% of all pregnancies. Several complications have been reported with this disease and it remains a major cause of maternal and fetal morbidity and mortality worldwide.[2] Unavailability of a precise test for identification of pregnant women at risk of developing preeclampsia is a major reason for the high morbidity of this disease.

A number of biochemical and haematological parameters change in preeclampsia in comparison to the normal pregnancy.[3,4] Creatinine, urea and uric acid are non-protein nitrogenous metabolites that are cleared from the body by the kidney following glomerular filtration. Measurements of plasma or serum concentration of these metabolites are commonly used as indicators of kidney function and other conditions.[5] Therefore, their determination in serum during pregnancy is of a major importance to diagnose kidney function especially in women with preeclampsia signs.

In 1917, the association between elevated serum uric acid and preeclampsia was reported for the first time.[6] From then, the uric acid measurement was considered as a component to monitor the severity of the disease in the pregnant women with preeclampsia. The degree of uric acid elevation correlates with the severity of proteinuria and renal pathological changes, and with fetal demise.[7]

Preeclampsia is associated with risk to the fetus which include intrauterine growth restriction, prematurity and death. Preeclamptic mother is at risk of renal failure, example acute kidney injury (AKI) or acute renal failure resulting from pre-renal azotemia, clinical manifestations of which tends to appear in preeclamptic women. The causes of AKI are often divided into three groups: pre-renal, intrarenal and post-renal. Pre-renal failure, also called prerenal azotemia, is described as a reversible increase in serum creatinine and urea concentrations which leads to a reduction in the glomerular filtration rate

(GFR). The BUN:Cr (Blood Urea Nitrogen to Creatinine) ratio is predictive of prerenal injury when BUN:Cr exceeds threshold value of 20. In prerenal injury, urea increases disproportionately to creatinine due to enhanced proximal tubular re-absorption.[8]

The literature suggests that no single marker is currently adequate to predict the development of preeclampsia and that a combination of indices would be most effective.[9] Increased plasma urea with normal creatinine concentrations giving rise to high ratios may be seen with any of the prerenal states.[10] Measurement of 24-hour urinary creatinine and protein excretion has several advantages as a diagnostic test being non-invasive, inexpensive, and easy to carry out. The use of serum uric acid and proteinuria as early predictors along with high BUN:Cr will help to identify pregnant females at high risk of preeclampsia and prompt the initiation of education and prophylactic interventions e.g. close prenatal care.[11]

The present study was undertaken to understand the changes in the biochemical parameters in serum and urine such as blood urea nitrogen, uric acid, LDH (Lactate dehydrogenases), platelet count, serum protein, 24 hour urinary creatinine and protein which would help in early diagnosis of preeclampsia and women at high risk of developing the disease and also to investigate the role of BUN:Cr as a preeclampsia predictor and as prognostic marker of underlying prerenal damage in preeclamptic women.

Materials and Methods

This cross sectional study was conducted from September 2012 to October 2013, in the Department of Clinical Laboratory Sciences, King Saud University and Section of Obstetrics and Gynecology, King Saud Medical City Hospital, Riyadh. The study was approved by hospital's ethics committee. Informed consent was obtained from patients before blood sampling.

The study comprised of 120 pregnant women divided into three groups- control (40) which included healthy normotensive pregnant women, 40 women at high risk of preeclampsia (HR group) and remaining 40 diagnosed with preeclampsia (PET group), attending antenatal OPD or labor room in their third trimester of pregnancy. HR group included pregnant women with mild hypertension, multiple gestation and gestational diabetes. The diagnosis of preeclampsia was based on the definition of American College of Obstetrics and Gynaecologists.[12]

On admission, venous serum samples were collected when the patients were in the supine position prior to commencement of intravenous therapy. At the time of blood collection, urine protein was measured by dipstick and was graded on a scale of 0 – 4+ (0: none; 1+: 30 mg/dl; 2+: 100 mg/dl; 3+: 300-1,999 mg/dl; 4+: at least 2,000 mg/dl). Blood samples obtained from patients attending OPD or admitted into hospital, were analyzed for complete blood count, measurement of serum creatinine, blood urea nitrogen, serum uric acid, LDH, platelet count, 24 hour urinary protein and creatinine.

All the biochemical parameters were determined in the biochemical analyzer, COBAS INTEGRA Autoanalyzer 800 (Roche, Germany). Serum Uric acid was measured by enzymatic colorimetric test ver., 2. Creatinine concentration was determined based on Jaffes reaction. Blood urea nitrogen (BUN) was measured using Urease test. Total protein concentration in serum and urine was determined using biuret Gene2 method in the COBAS INTERGRA 800 analyzer. Albumin in serum was measured using bromocresol green (BCG). Lactate dehydrogenases (LDH) was measured using IFCC liquid ver., 2. Haematocrit (Hct) concentration was measured in automated Cell Dyne 3700 analyser (Abbott, U.S) and platelet count was obtained using automatic reader, STA compact, Mediserv, UK.

Statistical Analysis: The results were expressed as Mean ± S.D. Statistical analyses were performed using SPSS software. Comparison of clinical characteristics and biochemical parameters of cases with control among the groups was performed by one - way ANOVA. The correlation between BUN:Cr ratio and various parameters were evaluated with the Pearson's correlation coefficient. $P < 0.05$ was considered to be statistically significant.

Results

The present study enrolled 120 pregnant women divided into three groups-control, HR and PET group. The mean and standard deviation values of the clinical characteristics of the control and cases are shown in Table 1. Age and haematocrit among control, HR group and PET group were not significantly different. Preeclamptic group has high gestational age compared to control and HR group. BMI of HR group (37.36 ± 9.005 kg/m^2) was found to be high compared to control and PET group (29.94 ± 6.05 and 35.12 ± 6.06 kg/m^2 respectively). The comparison of biochemical parameters within the three groups are represented in Table 2. BMI of HR group was significantly different from control and PET group. There was no significant difference in BMI between control and PET

group.

The mean value of systolic arterial blood pressure (sATP) of control group and HR group was 113.56 ± 13.93 mmHg and 124.70 ± 16.21mmHg respectively, while in PET group the sATP was 167.00 ± 24.43 mmHg. There was significant difference in the value of sATP ($p < 0.05$) among Control and HR group, and $p < 0.001$ was observed between control and PET group and between HR with PET group. The diastolic arterial blood pressure (dATP) was found to be high in PET group (98.51 ± 11.16) compared to control and HR group (67.66 ± 9.38 and 74.45 ± 19.14 respectively). We observed a significant difference in dATP between control and PET, HR and PET group ($p < 0.001$) and significant difference between control and HR group ($p < 0.05$).

The serum uric acid in PET group was significantly high compared to control ($p < 0.05$). Uric acid levels differ significantly ($p < 0.001$) between control and HR group and between HR and PET group. Serum creatinine levels were observed to be significantly high in PET group compared to control and HR group. There was significant increase in creatinine levels among HR and PET and between control and PET group ($p < 0.001$). On contrary, there was no significant difference between control and HR group.

Unlike serum creatinine, serum albumin was found to decrease significantly in PET group compared to control and HR group. The decrease in serum albumin levels was significantly different ($p < 0.001$) among all the groups studied. On the other hand, total serum protein was found to decrease significantly between control and HR group, control and PET group ($p < 0.001$) but there was no significant difference between HR and PET group.

In contrast to serum protein, the mean value of protein in 24 hour urine of PET group was found to be high compared to control and HR group. There was significant difference in urine protein of PET group with HR and control group ($p < 0.001$). There was no significant difference between control and HR group. Similarly the mean value of urinary creatinine increased significantly in PET group (7569.32 ± 224.82 µmol/l) compared to control and HR group (4058.42 ± 1353.39 and 5402.77 ± 1418.24 µmol/l respectively).The increase in levels of urinary creatinine was found to be statistically significant ($p < 0.001$) among all the groups. The mean value of LDH was found to be high in PET group compared to control and HR group. There was significant difference in LDH value among all the groups ($p < 0.001$).

Table-1: Mean and standard deviation values of the clinical characteristics in control, high risk (HR) and preeclampsia group (PET)

Characteristics	Control Group (n=40)	High Risk (HR) Group (n=40)	Preeclamptic Group (n=40)
Age (years)	31.20 ± 5.84	34.26 ± 6.69	31.55 ± 6.14
BMI (kg/m^2)	29.94 ± 6.05	37.36 ± 9.00	35.12 ± 6.06
Gestational Age (weeks)	31.17 ± 5.33	30.55 ± 6.33	33.72 ± 3.70
Haematocrit (%)	34.75 ± 4.30	34.48 ± 3.55	32.76 ± 3.71
Platelet Count (10^3/µl)	266.17 ± 84.83	209.82 ± 47.64	156.65 ± 52.21
sATP (mmHg)	113.56 ± 13.93	124.7 ± 16.21	167. 0 ± 24.43
dATP (mmHg)	67.66 ± 9.38	74.45 ± 19.14	98.51 ± 11.16
S. Uric Acid (µmol/l)	207.30 ± 56.85	346.05 ± 107.72	258.57 ± 83.82
BUN (mmol/l)	2.06 ± 0.78	3.91 ± 1.11	7.41 ± 0.66
S. Creatinine(µmol/l)	42.64 ± 9.58	45.21 ± 9.57	65.83 ± 18.50
S. Total Protein (g/l)	70.58 ± 5.91	59.74 ± 6.95	57.79 ± 6.71
S. Albumin (g/l)	33.10 ± 8.90	27.53 ± 4.76	22.07 ± 3.31
Protein (Urine) mg/day	198.60 ± 86.41	234.05 ± 127.27	1806.02 ± 777.6
24 hr Creatinine (urine) (µmol/day)	4058.42 ± 1353.3	5402.77 ± 1418.2	7569.32 ± 224.82
LDH (U/l)	201.27 ± 50.73	252.27 ± 57.43	360.95 ± 78.68
BUN:Cr	12.44 ± 4.14	22.47 ± 7.84	30.45 ± 9.073

Each value represents mean ± SD. BUN: Blood Urea Nitrogen; BUN:Cr: Blood Urea Nitrogen/Creatinine Ratio; sATP: Systolic Arterial Blood Pressure; dATP: Diastolic Arterial Blood Pressure

Table-2: Comparison of the clinical characteristics between three groups

Characteristics	Control Group (n=40)		High Risk (HR) Group (n=40)		Preeclamptic Group (n=40)	
	t	p	t	p	t	p
Age (years)	4.626	< 0.001*	3.23	0.003**	1.395	0.16
BMI (kg/m^2)	0.698	0.48	2.85	< 0.05**	2.15	0.06
Gestational Age (weeks)	0.31	0.75	1.98	0.096	2.30	0.06
Haematocrit (%)	3.964	< 0.001*	3.741	< 0.001*	7.705	< 0.001*
Platelet Count (10^3/µl)	2.63	0.01**	10.07	< 0.001*	12.64	< 0.001*
sATP (mmHg)	2.16	0.033**	7.66	< 0.001*	9.762	< 0.001*
dATP (mmHg)	7.26	< 0.001*	4.58	< 0.001*	2.68	0.008**
S. Uric Acid (µmol/l)	9.43	< 0.001*	17.80	< 0.001*	27.23	< 0.001*
BUN (mmol/l)	0.86	0.38	6.96	< 0.001*	7.83	< 0.001*
S. Creatinine(µmol/l)	7.40	< 0.001*	1.32	0.187	8.73	< 0.001*
S. Total Protein (g/l)	4.04	< 0.001*	3.94	< 0.001*	7.96	< 0.001*
S. Albumin (g/l)	0.34	0.73	15.36	< 0.001*	15.70	< 0.001*
Protein (Urine) mg/day	4.30	< 0.001*	6.93	< 0.001*	11.23	< 0.001*
24 hr Creatinine (urine) (µmol/day)	3.59	< 0.001*	7.66	< 0.001*	11.26	< 0.001*
LDH (U/l)	8.66	< 0.001*	6.89	< 0.001*	15.55	< 0.001*

* $p < 0.001$; ** $p < 0.05$ (Statistically significant); BUN: Blood Urea Nitrogen; BUN:Cr: Blood Urea Nitrogen/Creatinine Ratio; sATP: Systolic Arterial Blood Pressure; dATP: Diastolic Arterial Blood Pressure

Table-3: Pearson's Correlation coefficient of BUN/Creatinine ratio with various parameters

Characteristics	Control Group [r (p-value)]	High Risk (HR) Group [r (p-value)]	Preeclamptic Group [r (p-value)]
BMI	0.258 (0.10)	-0.07 (0.63)	0.14 (0.36)
Gestational Age	0.08 (0.59)	0.033 (0.83)	0.07 (0.64)
S. Uric acid	-0.09 (0.56)	0.083 (0.60)	0.039 (0.80)
S. Creatinine	-0.40 (0.009)	-0.58 (< 0.001)	-0.93 (< 0.001)
LDH	0.20 (0.20)	-0.16 (0.30)	-0.21(0.18)

The mean value of BUN:Cr in PET group (30.45 ± 9.073) or (30:1) was significantly higher ($p < 0.001$) compared to control and HR group (12:1, 22.4:1 respectively). There was significant difference between control and HR group, HR and PET, and between PET and control groups ($p < 0.001$). The levels of serum uric acid, platelet count and BUN:Cr, are shown in Figure 1. The overall significance was less than $p < 0.05$. Correlations of BUN: Creatinine ratio with various parameters were evaluated with the

Pearson's correlation, as shown in Table 3. There was positive correlation of BMI, gestational age and LDH levels and negative correlation of serum uric acid, creatinine with BUN:Cr in control group. Positive correlation of BUN:Cr with gestational age, uric acid in HR group, and negative correlation with creatinine, LDH in both HR and PET groups. The correlations of BUN:Cr with above parameters were not statistically significant.

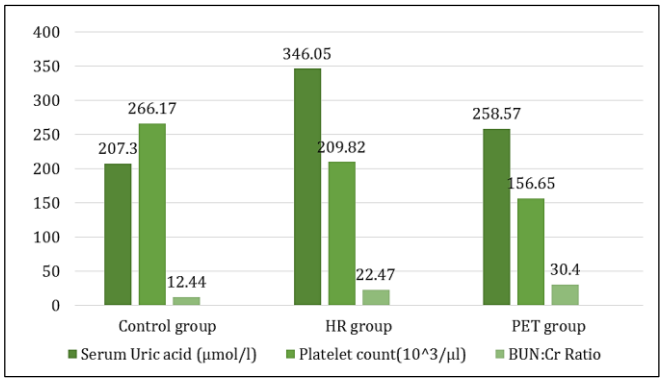

Figure-1: Serum uric acid, Platelet count and BUN:Cr ratio levels in Control, HR and PET Groups

Discussion

The present study comprised of pregnant women divided into three groups: normal healthy pregnant women, women at high risk of preeclampsia, and women with preeclampsia. The mean value of serum uric acid, blood urea nitrogen, creatinine, urinary protein and BUN: Creatinine ratio changed significantly in preeclampsia group compared to control group ($p < 0.001$). There was significant difference in serum uric acid between control and preeclampsia group ($p < 0.05$). However, there was no significant change in haematocrit, serum creatinine and urine protein between control and high risk group**.**

Hypertensive disorders of pregnancy complicate 5–10% of pregnancies and are associated with significant prenatal complications. Therefore, there is a constant search for markers that will be able to identify women who are at risk of developing preeclampsia during early pregnancy. There are several recognized markers that have been investigated in this context, however, most of the markers were found to be poor predictors of preeclampsia since they were not specific enough and/or were elevated only few weeks before preeclampsia developed.

Liver function abnormalities and renal function impairment are most important in causing complications like preeclampsia and associated early renal disease. During uncomplicated pregnancies serum uric acid concentration decrease by 25% to 35% in early pregnancy but then increase throughout pregnancy until towards the end of pregnancy when they approach non pregnant levels. It is proposed that these pregnancy mediated changes in serum uric acid are primarily the result of altered renal handling. Increased serum uric acid in preeclampsia is secondary to reduced renal uric acid clearance because of renal dysfunction.[9] It is also possible that increased serum uric acid values may indicate the presence of undiagnosed sub clinical renal disease in some subjects and this may increase the risk for preeclampsia.

In the present study, mean serum uric acid and creatinine levels were significantly higher in preeclampsia than in normal pregnancy. This could be due to increased re-absorption and decreased excretion of uric acid and creatinine in proximal tubules. The results obtained in the present study are consistent with previous reports.[13,14] On contrary, we observed increased serum uric acid levels in pregnant women at high risk of preeclampsia compared to normal and severe preeclamptic group, indicating the role of uric acid as a prognostic factor in development of severe preeclampsia.

BUN/Creatinine ratio in pregnant women with preeclampsia was significantly increased ($p < 0.001$) in comparison to the control group and HR group indicating the prerenal source of azotemia. High BUN:Cr in HR group compared to control is an important predictor identified in this study. This index can be important for the evaluation of preeclampsia severity. Total serum protein concentrations also increased significantly in women with preeclampsia compared to control. Higher levels of serum protein were observed in women at high risk compared to control and preeclampsia group. Compared with normal pregnancies, preeclampsia patients excrete significantly higher amounts of urinary protein. The increased albumin excretion in these patients appeared, on average, 9 weeks prior to the development of hypertension. In our study, we observed that higher urinary protein and BUN:Cr ratio in HR group could be identified as risk factors for preeclampsia and subsequent renal disorder. Levels of 24 hour urinary protein in PET group was increased significantly among all the groups and it is in agreement with previous reports.[15,16] The use of 24-hour urinary protein as early predictors will help to identify pregnant females at high risk of preeclampsia and prompt the initiation of education and prophylactic interventions (i.e. primary prevention e.g. close prenatal care).

A thorough research on all the biochemical parameters is thus necessary in understanding the pathophysiology and to identify biochemical markers that help in diagnosis of the disorder. This would help women at risk, in preventing

hypertension in subsequent pregnancies and later in their life. Our finding finally support that hyperproteinuria with hyperuricemia and high BUN:Cr ratio correlate to severe preeclampsia and increase in BUN:Cr ratio in HR group is an alarm for an effective prophylactic treatment to prevent the onset of renal damage in pregnant women. The index may be noteworthy in understanding the pathological stage of preeclampsia and helps in development of strategies for prevention and early diagnosis of maternal and fetal complications. Through our findings we suggest that pregnant women should be advised to have their blood pressure checked regularly to prevent future complications.

Conclusion

In conclusion, we observed that the levels of serum uric acid, protein, 24 hour urinary protein excretion along with BUN:Cr ratio increases significantly in women with preeclampsia. Increased levels of serum uric acid and protein act as efficient biomarkers for diagnosis of preeclampsia in pregnant women. Also the BUN:creatinine ratio serves as a predictor of preeclampsia and early diagnosis of prerenal damage associated with preeclampsia. However, further studies on a larger population needs to be undertaken to validate its sensitivity and specificity.

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