Sodium Imbalance in Preeclampsia

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Abstract

Background: Preeclampsia is a form of high blood pressure manifested during pregnancy. It is a common major complication causing significant morbidity and mortality; however, its etiology is unknown. Moreover, data on cation pattern during pregnancy are conflicting, and its relation with endothelial derived nitric-oxide and sex hormones have not been described adequately.

Objective: to demonstrate the pattern of sodium during preeclampsia with respect to normal pregnancy, and the correlation of the above parameter with nitric-oxide pathway.

Subject and methods: the present study is a cross-sectional case-control study includes measurement of nitric oxide NO), nitric oxide synthase (NOS) , serum and urinary sodium in 60 patients with preeclampsia. They were classified into two groups according to the gestational age:

- Preeclamptics in the second trimester G1: (n=30).
- Preeclamptics in the third trimester G2: (n=30,).

The results were compared with 60 apparently healthy pregnant women (as controls). They were classified according to the gestational age into two groups:

- Pregnants in the second trimester G3: (n=30).
- Pregnants in the third trimester G4: (n=30).

Results: showed a significant reduction in serum NO and NOS in the preeclamptics with significant increase in serum sodium accompanied by urinary retention of this cation (expressed as urinary sodium per urinary creatinine), as compared to the controls.

The regulatory effect of NO on fluid balance is supported by the positive correlation between NO and urinary sodium excretion indicating that NO had different effects on renal tubular reabsorption of sodium.

Conclusion: preeclamptics (in different gestational age groups) experienced vasospasm (manifested by low s.nitrite)s and altered sodium status when compared with healthy pregnant women matched with their age and gestational age.

Keywords:preeclampsia, nitric oxide , Sodium.

IRAQI J MED SCI, 2009; VOL.7 (1):41-48

Introduction

Preeclampsia is defined as the onset of hypertension and the presence of proteinuria during pregnancy, usually occurring after the 20th week of gestation in a previously normotensive woman and resolving completely by the sixth week after delivery of fetus [1, 2].

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Received: 21st July 2008, Accepted: 15th January 2009.

The pathophysiology of preeclampsia is thought to represent a defective response to the physiologic demands of normal pregnancy [2, 3]. Normal pregnancy is associated with profound changes in maternal homeostasis [4]. The endpoint of these changes is to provide the fetus with the necessary environment for growth and the mother with adequate protection against pregnancy complication [4].

Early modifications in the regulation of arginine-vasopressin and the rennin-angiotensin-aldosterone system are responsible for the increase in maternal plasma volume to the extent of 50% near term [4]. The mechanisms responsible for these important changes are still

incompletely understood. The principal determinant of extracellular fluid volume is sodium and it has been calculated that normal pregnancy is associated with the net retention of some 900 mmol (3- 4 mmol /L) of sodium. Net sodium retention during pregnancy appears in some ways paradoxical in that there is a marked increment in factors which are known to enhance nartriuresis [(5)]. These include glomerular filtration rate and circulating concentrations of progesterone and atrial natriuretic peptide. One noteworthy factor opposing this change is the very substantial increase in plasma aldosterone concentrations [(5)].

It is obvious that a significant proportion of the retained sodium must be sequestered within the fetal compartment (including placenta, membranes and amniotic fluid) and it is noteworthy that the mother plasma sodium concentration decreases slightly, implying that factors other than sodium retention may also be responsible for the water retention of normal pregnancy[(4)]. Substantial alterations have been described in intracellular water and electrolyte concentrations and it is possible that these relate to changes in cell metabolism[4]. Failure to achieve these adaptational changes has been associated with intrauterine growth restriction and hypertensive disorders in pregnancy [(4)].

Nitric oxide (nitrogen monoxide) plays an important role in a wide range of physiologic processes [(6)]. NO influences renal vascular tone and blood pressure (BP), glomerular and medullary hemodynamics, and extracellular fluid volume [(3)]. This renoprotective effect was supported by several genetic and experimental studies [(3)]. Nitric oxide synthase is particularly important in the function of human kidney. It plays a role in the maintenance of normal vascular and renal function [(7)]. Not surprisingly, renal signs and symptoms from inhibiting NOS are similar to those seen in preeclampsia [(7)]. There may be a similar nitric oxide generation and sodium ion relationship in the endothelial cells of the small intestine and the tubules of the kidney cells in that when they are stressed by sodium entry, the exchange of sodium for calcium activates calcium dependent NOS[(8)]. A link between tubular absorption of sodium ions and NO generation has been shown in both in vivo and in vitro preparations [(7)].

Subjects & Methods

A-Subjects

The study was a cross-sectional, case-control study conducted on 60 patients with preeclampsia (PE) attending the Obstetric Consultant-Clinic, Antenatal Clinic, and Labor Ward at Al-Kadhimiya Teaching Hospital, for re-evaluation of newly diagnosed PE, or for delivery.

The diagnosis of PE was based on clinical criteria that were hypertension (absolute BP of 140/90 mmHg twice over 4 hr without prior comparison) [(1, 2)] and proteinuria (21.5 mg of urinary protein per µmol creatinine) [(9)].

The exclusion criteria used for cases and controls were gestational or chronic hypertension, diabetes mellitus, renal disease, multifetal gestation, intrauterine fetal death, and pregnancy less than 20 weeks of gestation.

Depending on the gestational age, the 60 patients were divided into two groups:

1. Preeclamptics in the second trimester **(G1):** They were 30 with age range from 18 to 37 years (mean age $\pm$ SD = 26.1 $\pm$ 6.4 year) and gestational age range from 20 to 28 weeks (mean gestational age $\pm$ SD = 26.3 $\pm$ 1.5 week).

2. Preeclamptics in the third trimester

(G2): They were 30 with age range from 18 to 40 year (mean age ± SD = 25.1 ± 6.9 year), and gestational age range from 29 to 40 weeks (mean gestational age ± SD = 35.6 ± 1.6 week)

The study included another 60 apparently healthy pregnant women attending the Antenatal clinic, and Labor Ward at Al-Kadhimiya Teaching Hospital, for re-evaluation of their pregnancy, or for delivery. They were included as normal controls. They were comparable with preeclamptic groups regarding the age and the gestational age. They were divided into two groups according to their gestational age:

1-.Normal pregnant women in the second trimester **(G3):** They were 30 with age range from 15 to 38 years (mean age ± SD = 24.6 + 4.5 year), and gestational age range from 20 to 28 weeks (mean gestational age ± SD = 25.5 ± 1.8 week).

2-Control pregnants during the third trimester **(G4):** They were 30 with age range from 18 to 35 year (mean age ± SD = 24.8 ± 4.6 year) and gestational age range from 29 to 40 weeks (mean gestational age ± SD = 34.6 ± 2.1 week).

B. Blood & urine samples:

Ten milliliters of random venous blood were withdrawn from each patient and control, in supine position, without application of tourniquet. Samples were transferred into clean new plane tube, left at room temperature for 15 minutes for clotting, centrifuged, and the separated sera were, then, divided into two parts:

1) An aliquot of serum was transferred into Eppendrof tube, which was used for measuring nitric oxide expressed as nitrite (the end product of NOS), this was done at the same day of collection [(10)].

2) The rest of serum was transferred into Eppendrof tube and was used for measurement of electrolytes (Na, K) [(11)]. The tubes were stored at –20° C until analysis, which was done within one month after collection [(11)].

Random urine specimens were obtained from each subject in the study to quantify urinary sodium and potassium [(11)] that was expressed as a ratio to the urinary creatinine [(11)].

As a preservative, 1-2 mls of 6M HCl was added to each random urine specimen; the samples were stored in appropriate containers at -20ºC until analysis within one month after collection [(11)].

C-Methods

Nitrite concentration measurement can be used as an index of NO synthase activity [(10)], this basic principle was used throughout the study. NO synthase activity is expressed here as the amount of nitrite (in µmoles) formed per minute, whereas the specific enzyme activity was given as the amount of nitrite (in µmoles) formed per minute per mg of protein for plasma [(10)] (µmol/min/mg protein). Serum and urinary sodium and potassium were analyzed by atomic absorption spectrophotometer [(11)].

Results

Serum Nitric oxide (NO) and nitric oxide synthase (NOS):

In preeclamptic pregnants in the third trimester G2, the maternal serum NO and NOS levels were significantly lower than those in the second trimester G1 [P< 0.001 for NO, < 0.05 for NOS]. In preeclamptic pregnants G1 & G2, the maternal serum NO and NOS were significantly lower than healthy pregnants G3 & G4 [P< 0.001 for both parameters & both groups], this difference was not found between healthy pregnants in second trimester G3 nor in third trimester G4 [P>0.05 for both parameters] as in Table 1.

Serum sodium (Na):

Serum sodium was significantly elevated in the preeclamptics (G1 & G2) with respect to their controls (G3 & G4) [P< 0.001 for the second trimester groups, < 0.05 for the third trimester groups]. Moreover, serum sodium was significantly increased in the third trimester healthy pregnant group G4 when compared with the second trimester pregnant group G3 [P= 0.01], but serum sodium was insignificantly decreased in the third trimester preeclamptic group (G2) when compared with the second trimester preeclamptic group G1 [P= 0.1] as in Table 2.

Urinary excretion of sodium expressed as sodium: creatinine ratio was significantly reduced in preeclamptics G1 and G2 when compared to corresponding controls G3 and G4. This reduction was also seen when second trimester pregnants in G3 was compared with third trimester pregnants in G4; however, the reduction in sodium excretion in third trimester preeclamptics G2 did not reach to a statistically significant level when compared with second trimester preeclamptics G1 as in Table 2.

Correlation between urinary sodium and serum NO:

A significant positive correlation between urinary sodium and serum NO level was noticed in different studied groups: in preeclamptics G1 and G2 (r=0.8, P < 0.001; r=0.8, P < 0.001) respectively and in pregnant control groups G3, and G4 (r=0.8, P < 0.001; r=0.8, P < 0.001) respectively as in Figures 1, 2, 3, and 4.

Table 1: The mean NO concentration (expressed as nitrite) and the mean NOS activity (expressed as nitrite formed per g protein per minute) in sera of different preeclamptic and control pregnant groups (presented as mean ± SD).

Variable	G1	G2	G3	G4
Nitric oxide (µmol)	6 ± 0.9	4.1 ± 2.4	8.1 ± 3	8.8 ± 3.3
NOS (µmol/g/min)	0.08 ± 0.01	0.06 ± 0.03	0.1 ± 0.04	0.11 ± 0.04

Table 2: The mean sodium values in serum and urine (expressed as urinary sodium per creatinine) of different preeclamptic and pregnant control groups (presented as mean ± SD).

Variable	G1	G2	G3	G4
Serum sodium (mmol/L)	140.9 ± 2.3	139.9 ± 2.3	136.5± 1.6	138.3 ± 3.6
Urinary sodium : creatinine	12.6 ± 6.9	11.2 ± 8.5	38.3 ± 9.4	47.7± 15.1

G1 & G2: *Preeclamptics in the second & third semesters,*
G3 & G4: *normal pregnants in the second & third semesters.*

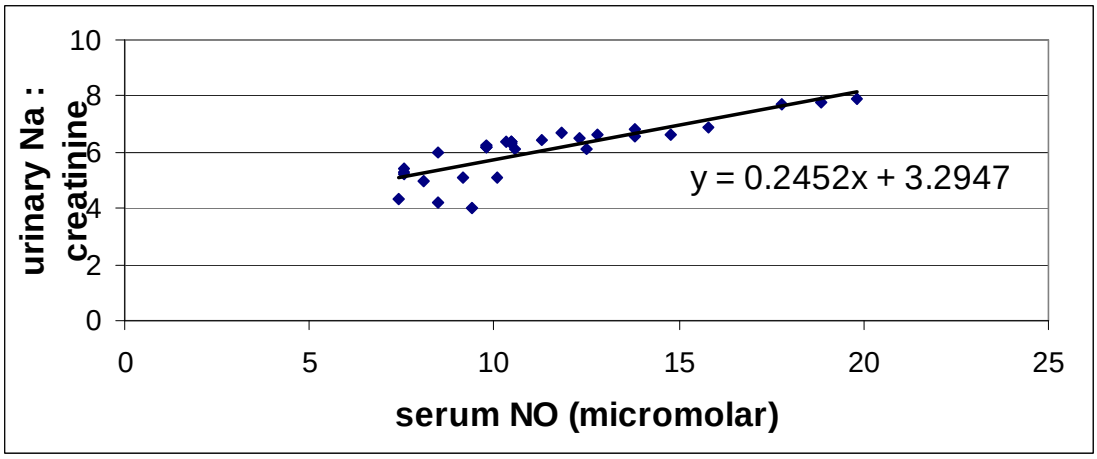

Figure 1: Correlation between serum NO & Na excretion in G1: second trimester preeclamptics (n=30; r = 0.8; P< 0.001).

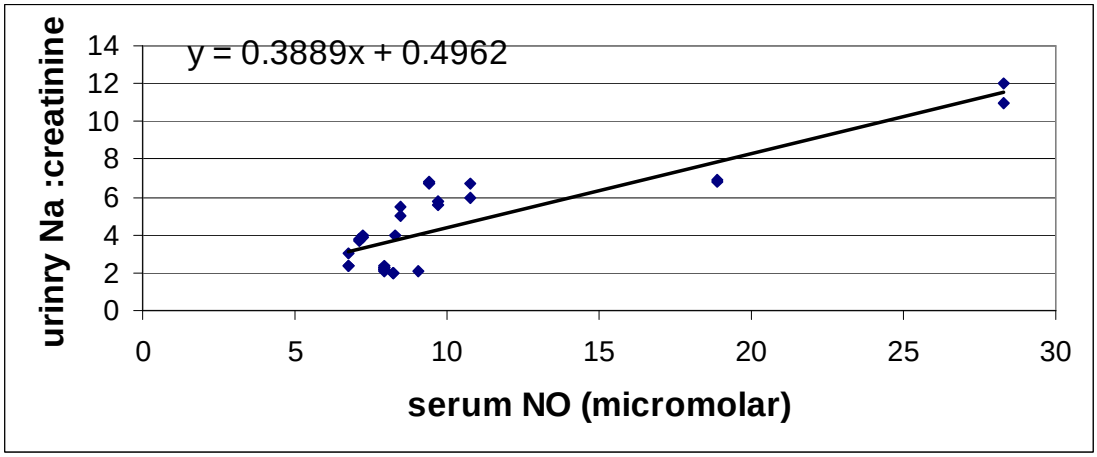

Figure 2: Correlation between serum NO & Na excretion in G2: third trimester preeclamptics (n=30; r = 0.8; P< 0.05).

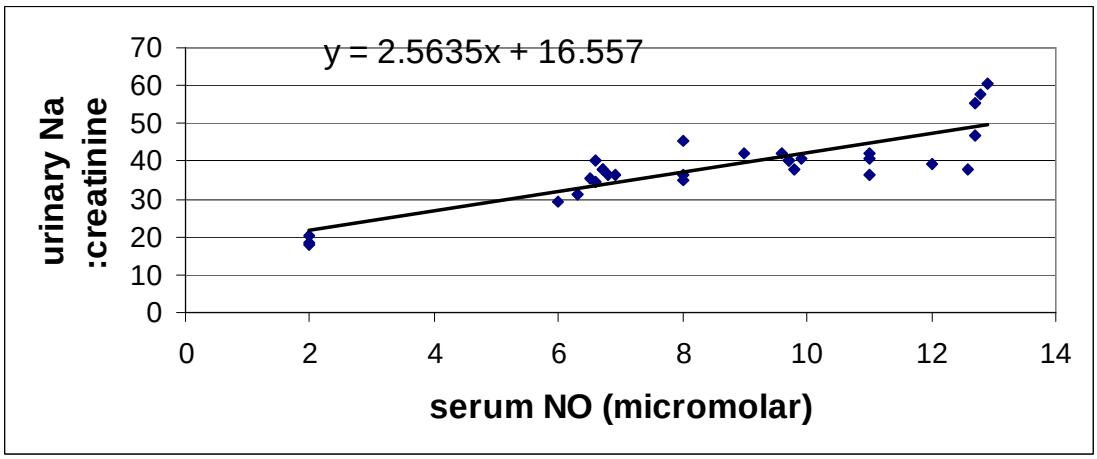

Figure 3: Correlation between serum NO & Na excretion in G3: second trimester pregnant controls (n=30; r = 0.8; P < 0.001).

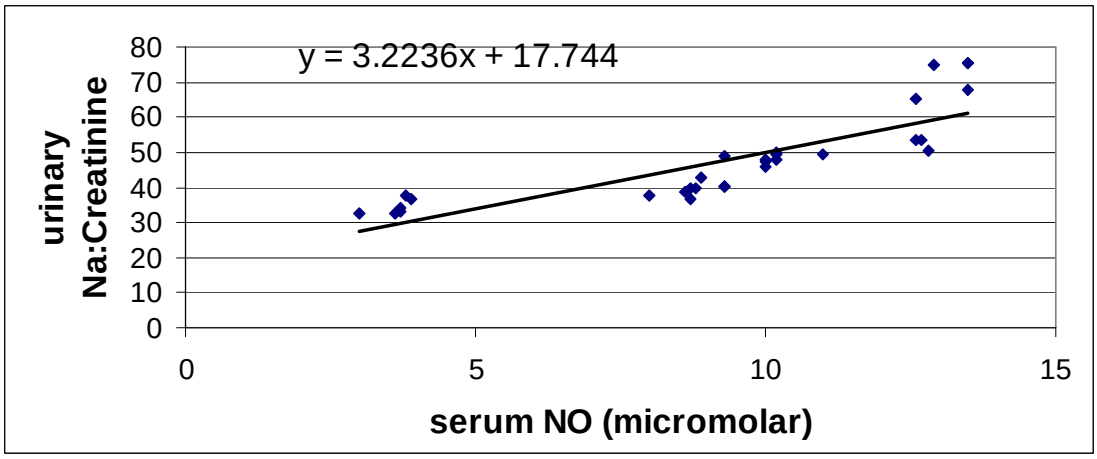

Figure 4: Correlation between serum NO & Na excretion in G4: third trimester pregnant controls (n=30, r = 0.8; P < 0.001).

Discussion

Nitric oxide mediates many functions of endothelium, including vasodilatation and inhibition of platelet aggregation [12]. Preeclampsia may be associated with nitric oxide deficiency [12], and the results of this study provide an evidence to support this hypothesis. As shown in Table 1. NO level in blood was similar in both healthy pregnants groups; it was unchanged during physiological pregnancy. During preeclampsia, the NO was decreased compared to the control level. This suggests that during preeclampsia the low activity of endothelial NO-synthases and redox-dependent transformation of NO in peroxynitrite provoke a decrease in the blood nitric oxide level [13], these results are comparable to those of Meher & Duly [12], Khetsuriani et al. [14], Choi et al.[13], Nishikawa & Miyamoto[15]

While serum Na^+ was significantly increased in normal pregnancy with advancing gestational age, it was insignificantly decreased in preeclamptics with advancing gestational age.

The observed significant low urinary excretion of sodium in the preeclamptic groups (Table 2) is comparable with Martniz et al. [16], who found that urinary excretion of sodium was lower in hypertensive than in normotensive gestation. But this finding can not be compared with the results of Halhali et al. [17], Kyey`nska et. al. [18], & Sigurdsson & Gengtss[19] who found normal range of urine Na excretion in their patients.

Preeclampsia is accompanied by amplification of the sodium retention that is a feature of a normal pregnancy [20]; which is associated with net retention of sodium with substantial alterations in intracellular water and electrolyte concentrations and possibly these are related to changes in cell membranes [21], which appear to be responsible for some pathological changes in preeclampsia. Some of the best documented alterations involve changes in the handling of sodium ion both on the systemic and intracellular levels [20, 22].

On intracellular level majority of studies support an increase in peripheral cell sodium concentration. This would suggest a defect in Na,K ATPase or sodium pump activity, leading to an increase cell sodium in vascular tissues that has been shown to enhance vascular sensitivity to vascular constricting agents or leading

directly to increased vasoconstriction [20,22].

While on the systemic level it has been suggested that blood volume depletion with subsequent reduction in the glomerular filtration rate can lead to Na retention [23]. Moreover, there is a broad agreement that component of renin-angiotensin-aldosteron pathway are markedly reduced in women with preeclampsia [16].

In this study, high serum sodium and low urinary sodium and their relation to low NO level in preeclampsia can be interpreted by understanding the role of NO in the regulation of sodium and fluid transport in the proximal tubule[24], NO functions as an inhibitor for the proximal tubular fluid and sodium reabsorption[24]. In this sense, NO is a natriuretic agent [24]. This is, in principle, consistent with the prominent role of NO in maintaining vascular tone and preventing increase in blood pressure [24]. However, the final effect of NO on proximal tubular sodium reabsorption and its role in the overall fluid and electrolyte homeostasis may vary under different circumstances [24]. The final effect of NO on proximal tubular reabsorption appears to depend on the concentration of NO and involve interaction with other regulatory mechanisms [24]. This is mainly caused by the complex effect of NO on various targets, including hemodynamics, the renin-angiotensin system, and the tubular system [24]. The above facts were confirmed by the positive correlation found between NO and sodium levels in both preeclamptics and control pregnants as seen in Figures: 1, 2, 3, and 4.

Biochemical changes in preeclampsia appear to be driven by a reduction in nitric oxide synthesis (as evident by low serum nitrite). This will, in turn, results in changes involving electrolyte metabolism and appearance of the typical pattern which may cause vasospasm of eclampsia. These changes would include relative increase in serum sodium with a reduction in its urinary excretion. These manifestations are evident by the existence of positive correlations between the parameters studied. Further study of the relation between sodium excretion and NO production by renal tissues is required. Also, Study of the membrane Na^+, K^+ ATPase and calcium pumps; as abnormalities of these pumps are also involved in the pathogenesis of preeclampsia.

References

1. Baker PN. (Ed.). Obstetrics by Ten Teacher; 18th edition. 2006; P: 159-161. Hodder Arnold

2. Parry S, Marchiano D. Hypertension in pregnancy. In: Mark-M and Sam-S. (Eds.). NMS (National medical series for independent study) /Obstetrics & gynecology. 5th ed. 2005; P: 169. Lippincott Williams & Wilkins.

3. Hollenberg ND. Organ systems dependent estrone nitric oxide and the potential for nitric oxide-targeted therapies in related diseases.*The Journal of Clinical Hypertension. 2006*; ***8 suppl4****: 63-73.*

4. Kametas N, McAuliffe F , Krampl E , Sherwood R, Nicolaides KH. Maternal electrolytes addition liver function changes during pregnancy at high altitude. *Clinica Chimica Acta. 2003;* ***328****: 21-29.*

5. Dunlop W, Normal pregnancy: physiology and endocrinology. In: Edmonds-DK. (Eds.). Dewhurst Textbook of Obstetrics and Gynecology for postgraduates. 6th ed. 1999; PP: 81-3. Blackwell Science.

6. Giles TD. Organ systems dependent on nitric oxide and the potential for nitric oxide-targeted therapies in related diseases. *The Journal of Clinical Hypertension. 2006;* ***8 suppl4****: 2-16.*

7. Lowe DT. Nitric oxide dysfunction in the pathophysiology of preeclampsia. *Nitric oxide: biology and chemistry. 2000;* ***4****; 4441-58.*

8. Kempson S, Thampson N, Pezzuto L, Bohlen HG. Nitric Oxide Production by mouse renal tubules can be increased by a sodium-dependent mechanism. *Ntric Oxide. 2007;* ***17****:33-43.*

9. Yamasmit Water, Chaithongwogwatthana S, Charoenvidhya D, Uerpairojkit B, Tolosa J. Random urinary protein-creatinine ratio for

prediction of significant proteinuria in women with preeclampsia. *J-Matern-Fetal-Neonatal-Med. 2004; **16**:257-9.*

10. Rachmilewitz D, Stamler JS,Bachwich D, Karmeli F, Ackerman Z, Podolsky DK. *Gut.1995. **36**:718-23.* Cited from Murshed A.Q. M. Mohammed. Study on nitric oxide synthase in kala-azaric patients. MSc. thesis. 1999. College of Science. Baghdad University.

11. Endres DB, Rude RK, Mineral and Bone Metabolism. In: Carel-AB, and Edward-RA. (Eds.). Tietz Textbook of Clinical Chemistry. 3rd ed. 1999; P: 1395-1412. Saunders Company, Philadelphia.

12. Meher S, Duly L. Nitric oxide for preventing preeclampsia and its complications. *Cochrane Database Syst Rev. 2007; **18**: CD006490.*

13. Choi JW, Im MW, Pia SH. Nitric oxide production increases during normal pregnancy and decreases in preeclampsia. *Ann Clin Lab Sci. 2002; **32**: 257-63.*

14. Khetsuriani T, Chabashvili N, Sanikidze T. Role of endotheline-1 and nitric oxide level in pathogenesis preeclampsia. *Georgian Med News. 2006; **141:** 17-21.*

15. Nishikawa S, Miyamoto A, Yamamoto H, Ohshika H, Kudo R. The relationship between serum nitrite and endothelin-1 concentrations in preeclampsia. *Life-Sci. 2000; **67**: 1447-54.*

16. Martniz AE, González OM, Grover PF, Vera Hernández A. Excretion of uric acid, sodium, and potassium in preeclampsia patients and its behavior in acute hyperglycemia-hyperinsulinemia.*Ginecol-Obstet-Mex. 1999; **67**: 590-4.*

17. Halhali A, Diaz L, Avila E, Ariza AC, Garabédian M, Larrea F. decreased fractional urinary calcium excretion and serum 1,25-dihydroxy vitamin D and IGF- I levels in preeclampsia. *J-Steroid-Biochem-Mol-Biol. 2007; **103**: 803-6.*

18. Kuezyǹska SJ, Wòjcicka JJ, Romejko E, Siekierski BP. Kidney function in women with pregnancy-induced hypertension. *Ginekol-Pol. 1989; **60**: 271-5.*

19. Sigurdsson JA, Gengtsson C. Urinary findings and renal function in hypertensive and normotensive women. *Acta-Med-Scand-Supppl. 1981; **646**: 51-3.*

20. Walker BR, Williamson PM, Brom MA, Honor JW, Edwrds CR, Whiteworth JA. 11-β-Hydroysteroid dehydrogenase and its inhibitors in hypertensive pregnancy. *Hypertension. 1995; **25:** 626-30.*

21. Dunlop W. Normal pregnancy: physiology and endocrinology. In: Edmonds-DK. (Eds.). Dewhurst Textbook of Obstetrics and Gynecology for postgraduates. 6th ed. 1999; PP: 81-3. Blackwell Science.

22. Graves SW. Sodium regulation, sodium pump function and sodium pump inhibitors in uncomplicated pregnancy and preeclampsia. *Front Biosci. 2007; **1:2438**-46.*

23. Kashyap MK, Saxena SV, Khullar M, Sawhney H, Vasishta K. Role of anion gap and different electrolytes in hypertension during pregnancy (preeclampsia). *Mol Cell Biochem. 2006; 282: 157-167.*

24. Liang M, Knox FG. Production and functional role of nitric oxide in the proximal tubule. *Am-J-Physio-Regul-Integr-Comp-Physiol*. 2000; ***278**: R1117-R1124.*