American Journal of Biomedical and Life Sciences
2019; 7(4): 93-98
http://www.sciencepublishinggroup.com/j/ajbls
doi: 10.11648/j.ajbls.20190704.14
ISSN: 2330-8818 (Print); ISSN: 2330-880X (Online)

A Study to Assess the Significance of Urinary Collagen IV Excretion to Predict Preeclampsia in Early Pregnancy

Kaneez Fatema[1, *], Fatema Jebunnesa[2], Salima Akter[3], Towhidul Alam Chowdhury[4], Liaquat Ali[3]

[1]Deptment of Obstetrics and Gynecology, Faridpur Medical College, Faridpur, Bangladesh

[2]Deptment of Biochemistry and Cell Biology, Bangladesh University of Health Sciences (BUHS), Dhaka, Bangladesh

[3]Deptment of Medical Biotechnology, Bangladesh University of Health Sciences (BUHS), Dhaka, Bangladesh

[4]Deptment of Obstetrics and Gynecology Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, Bangladesh

Email address:
moyourakhi2003@yahoo.com (K. Fatema)

[*]Corresponding author

To cite this article:
Kaneez Fatema, Fatema Jebunnesa, Salima Akter, Towhidul Alam Chowdhury, Liaquat Ali. A Study to Assess the Significance of Urinary Collagen IV Excretion to Predict Preeclampsia in Early Pregnancy. *American Journal of Biomedical and Life Sciences*. Vol. 7, No. 4, 2019, pp. 93-98. doi: 10.11648/j.ajbls.20190704.14

Received: November 7, 2018; **Accepted**: August 22, 2019; **Published**: September 10, 2019

Abstract: It was a prospective study with a case control design. The purpose of the study was to measure the urinary collagens IV (U-coll IV) and microalbumin (MA) level in early pregnancy and to explore the role of excretion U-coll IV in prediction of preeclampsia (PE). A total number of 119 pregnant women at 10-14 weeks of pregnancy were selected on the basis of availability. MA by immunoturbidimetry assay and U-coll IV by enzyme immunoassay method were measured in these subjects and they were followed up to the term for the possible development of PE. MA was defined by albumin-creatinine ratio (ACR) above 32mg/g and high U-coll IV was defined by values above the cut-off point 2.74 ng/ml determined by the median value of the (controls). The data were analyzed by grouping the subjects who developed PE in later stages of pregnancy (the PE group) and those who did not develop PE in later stages (Control group). From the total subjects, 10 developed PE which shows a prevalence of about 8.4%. The PE group had higher value of ACR as compared to Control [ACR, mg/g, median (range), 42.3]. The sensitivity of ACR in predicting PE was 80%, specificity 49.54%, PPV 12.69% and NPV 96.42% respectively. The sensitivity of high U-coll IV in predicting PE was 70%, specificity 50.5%, PPV 11.5% and NPV 94.8%. It may be concluded that early pregnancy levels of MA and U-coll IV can be used as predictors of PE with high negative predictive value; and U-coll IV has no added advantage over MA in this respect.

Keywords: Urinary Collagen IV, Preeclampsia, Microalbuminuria

1. Introduction

Preeclampsia is a pregnancy-related hypertensive disorder occurring usually after 20 weeks of gestation. If left untreated, it progresses to eclampsia [1]. Preeclampsia has remained a significant public health threat in both developed and developing countries contributing to maternal and perinatal morbidity and mortality globally [2-5]. Worldwide, the incidence of preeclampsia ranges between 2% and 10% of pregnancies. WHO estimates the incidence of preeclampsia to be seven times higher in developing countries (2.8% of live births) than in developed countries (0.4%) [6]. Preeclampsia is not only common and dangerous for both mother and baby, but also unpredictable in onset and progression, and is incurable until termination of the pregnancy. PE is the second leading cause of maternal mortality constituting 12% to 18% of pregnancy related maternal deaths [7]. PE is known as 'the disease of multiple theories'. Among them genetic, immunological, circulatory factors, uterine vascular changes and endothelial dysfunction are important [8].

Predisposing factors are nulliparity, black race, maternal age below 20 or over 35 years, low socioeconomic status, multiple gestation, hydatidiform mole, polyhydramnios,

nonimmune fetal hydrops, twins, obesity, diabetes, chronic hypertension and underlying renal disease [8].

Investigators have been evaluating tests to predict preeclampsia for over 50 years without much success [9, 10]. The list is enormous and these have included "roll over" tests, cold pressor tests, uterine artery Doppler evaluation tests, pro- and anti-angiogenic proteins, hCG, placental protein 13, inhibin A, fibronectin, P- and L-selectin, VCAM-1, fractional urate clearance, microalbuminuria etc. A large systemic review published in 2004 [9] concluded that no single test met clinical standards for a predictive test.

The pathophysiological events resulting in pre-eclampsia begin early in gestation, and precede the onset of the clinical features [11]. One of the early pathophysiological hallmarks is endothelial cell damage [12, 13]. Microalbuminuria, have been proposed as useful integrated markers of sub-clinical target organ damage and renal endothelial injury resulting from local or systemic vascular damage. Microalbuminuria is characterized by urinary albumin excretion above normal levels in the absence of clinically detectable nephropathy [14-16]. Microalbuminuria is noted to be present if urinary albumin is within the range of 30-300 mg/24 hrs [17]. Studies have shown that the presence of microalbuminuria earlier in pregnancy is associated with an increased risk of development of PE and severe adverse maternal and fetal outcome in PE [15, 16]. Microalbuminuria is one the classic signs of preeclampsia. The presence of microalbuminuria in some otherwise symptom-free patient confirms that changes in renal function are present in whom pre-eclampsia would eventually develop.

Microalbuminuria is a marker of endothelial dysfunction and is also the only clinical sign of later developing diabetic nephropathy. As collagen IV is a principal component of glomerular basement membrane and mesengial matrix, many studies have identified the levels of collagen IV in serum and urinary samples are more sensitive index of early stage of glomerular injury in patients with diabetic nephropathy [18, 19]. Renal function changes in PE have been documented and several prospective studies indicate that at least some of these changes are present before the clinical diagnosis of PE [20]. However, none of the previous studies have measured urinary collagen IV in pregnant women. In this study a group of pregnant women have been investigated to explore the suitability of urinary collagen IV and ACR as predictive markers at early stage of pregnancy (10-14 wks).

2. Methods

In this prospective study, total number of 119 pregnant women at 10-14 weeks of pregnancy was selected on the basis of availability from the antenatal clinics of BIRDEM, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka Medical College Hospital (DMCH) and from private clinics of the specialists in Obstetrics & Gynecology. Any pregnant woman having history of chronic hypertension, diabetes, renal disease, multiple pregnancies, urinary tract infection and any acute or chronic illness were temporarily excluded from the study. The study was conducted at the Department of Obstetrics & Gynecology and at the Department of Cell & Molecular Biology, Research Division, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM). The purpose of the study was explained to each individual subject and informed written consent was taken.

2.1. Subjects Collection Technique

At the time of registration, a detailed obstetric and medical history was taken by following a pre-designed data sheet. Women with presence of hypertension (BP >140/90mmHg) with proteinuria (using by dipstick method), women with history of hypertension (as evidenced by medication), diabetes, renal disease and multiple pregnancies were excluded from the study. Preeclampsia was diagnosed when the systolic blood pressure ≥140 mmHg and/or diastolic BP ≥90 mmHg on two occasions at least 6 hours apart, developing after 20 weeks of gestation in previously normotensive women with proteinuria greater than 300 mg or more in 24 hours or greater or 2 readings of at least ++ on dipstick analysis of mid-stream specimen of urine [8]. [According to American College of Obstetrics & Gynecology (ACOG)]. Women with urinary tract infection were temporary excluded. General, systemic and obstetric examinations were carried out on the same day. These patients were under regular follow up until delivery. Specific note was made of the development of preeclampsia/eclampsia during antenatal period and/or at the time of delivery.

2.2. Sample Collection Procedure

Clean catch fasting morning urine sample (5 ml) were collected in a clean container from all the selected subjects during their 10 to 14 weeks of pregnancy, then urine sample for collagen IV assay was kept in a specific test tube for urinary collagen IV with preservative (provided by Biotrin International, Ireland). Urine for microalbumin was preserved in a separate appendrop and both were preserved at -70°C until biochemical analysis was performed. Serum was separated rapidly after centrifuging for 10 minutes at a rate of 3000 rpm at 4°C and then preserved divided into different aliquots and frozen at -70°C until biochemical analysis was done.

2.3. Statistical Analysis

Statistical analysis was performed using a statistical package (SPSS for Windows). Data was expressed as mean±SD or median (range) as appropriate. The statistical significance of differences was assessed by Student's unpaired t-test, Mann Whitney U-test and Chi square test, where appropriate. McNamara chi square test was used to calculate sensitivity, specificity, PPV and NPV. The difference between groups were evaluated with the "p" value <0.05.

2.4. Lab Analysis of Microalbumin and Collagen IV

Esimation of serum/urinary creatinine was done by alkaline-picrate method using reagents of Randox Laboratories, UK. The urinary microalbumin (MA) concentration was determined by immunoturbidimetry assay [21]. The urinary Collagen IV assay was done by enzyme immunoassay method [22] with a research kit (The Biotrin Urinary Collagen IV EIA kit).

3. Results

In the study, among 119 women PE developed in 10 patients, of whom 4 were primigravida and 6 were multigravida. The rest 109 patients remained normotensive (Controls). The prevalence of PE among the study group was 8.4%. No statistically significant difference was found between case and controls regarding maternal age, body mass index and gestational age. The SBP (mean±SD, mmHg) were 105±11 vs 134±11, DBP (mean±SD, mmHg) 67±9 vs 92±10 and MAP (>80 mmHg) 80±8 vs 106±9 respectively in Control and PE subjects. The entire BPs was higher in PE cases and the differences with the Control were highly significant ($p<0.001$).

Table 1. *Baseline characteristics of the Study Subjects.*

Characteristic	Control group (n=109)	Preeclampsia (n=10)	P value
Age (yrs)	25±4	26±5	0.68/0.499
BMI (kg/m²)	22.68±3.49	24.54±4.35	1.58/0.111
SBP (mmHg)	105±11	134±11	7.7/0.001
DBP (mmHg)	67±9	92±10	8.80/0.001
MAP (mmHg)	80±8	106±9	9.30/0.001
S Creat (mg/dl)	0.80±0.10	0.80±0.08	0.01/0.984

Results were expressed as mean±SD; n, number of subjects. p value was calculated using by Student's unpaired t-test.

3.1. Preeclampsia Among Microalbuminuric and High Collagen IV Subjects

The cut-off value of ACR for microalbuminuria was taken as 32mg/g creatinine from McCormik (1990) [23]. Since there is no published data on the reference range of urinary collagen IV in PE patients, we took median of urinary collagen IV in our Controls.

3.2. Microalbumin Status of the Study Subjects

The value of ACR [mg of albumin/ g of creatinine] median (range) was 33.33 (6.7-98.5) in Control subjects whereas the corresponding value was 42.38 (28.8-63.05) in PE subjects. The ACR value of PE subjects was relatively higher than that of the controls.

3.3. Urinary Collagen IV Status of the Study Subjects

The median (range) value of collagen IV was 2.54 (0.80-9.85) ng/ml in Control subjects and the corresponding value was 2.78 (1.60-7.50) in PE subjects. The median value showed no significant differences (Table 2).

Among total ACR positive 63 subjects 87.30% were control and 12.69% were PE. Among collagen IV positive 61 subjects 88.53% were control and 11.47% were PE. Among 10 PE subjects 8 had high ACR and 2 had normal level. On the other hand, 7 PE subjects had high and 3 had normal level of urinary collagen IV.

Table 2. *Serum Creatinine, Urinary Albumin/Creatinine ratio and Urinary Collagen IV levels in the Study Subjects.*

Variable	Control (n=109)	PE (n=10)	t/p or U/p value
S Creat (mg/dl)	0.80±0.10	0.80±0.08	-0.01/0.98
ACR (mg/g)	33.33 (6.7-98.5)	42.38 (28.8-63.05)	384.0/0.074
Coll-IV (ng/ml)	2.54 (0.80-9.85)	2.74 (1.60-7.50)	433.0/0.217

Data were expressed as Mean±SD for serum creatinine and in Median (range) for ACR and urinary collagen IV. t/p value was calculated using by Student's unpaired t-test; U/p value was calculated using Mann-Whitney U test.PE, Preeclampsia; S creat, Serum creatinine; ACR, Albumin Creatinine Ratio; Coll-IV, Urinary collagen IV

Table 3. *Predictive values of Collagen-IV and Microalbumin at different percentile in the study subjects (n=119).*

Variable	Percentile	Cut-off Value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Collagen-IV level	25th	1.83	90	26	10	97
	50th	2.62	70	51	12	95
	75th	3.85	30	75	10	92
Microalbumin	25th	16.5	80	25	9	93
	50th	26.4	40	48	7	48
	75th	51.8	30	75	10	92

Data were express as sensitivity; specificity calculated by *McNamara* chi squire test.

3.4. Association of PE with Various Baseline Parameters as Explored by Binary Logistic Regression (Table 3)

The ROC curve for the microalbumin level in urine is shown in Figure 1. The area under the curve is 0.689 (95% confidence interval is 0.566 and 0.811). The sensitivity, specificity, PPV and NPV for various cut offs are shown in the Table. The cutoff of ≥ 16.5 yields a sensitivity of 80% and a specificity of 25%. With the use of this cutoff, there were 2 false-negative test results and 82 false-positive test results.

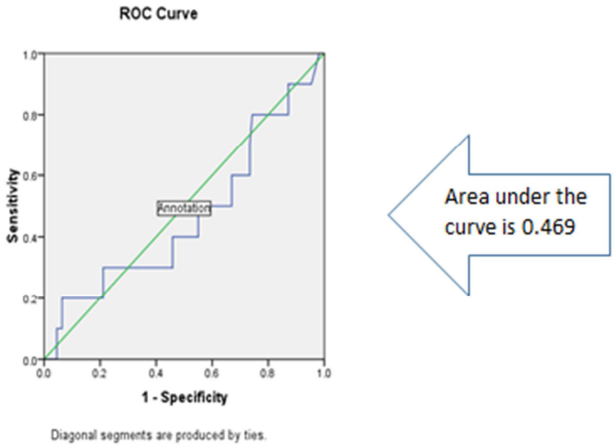

Figure 1. *ROC curve for various cutoffs of microalbumin level as a predictor of preeclampsia.*

The ROC curve for the collagen level in urine is shown in Figure 2. The area under the curve is 0.611 (95% confidence interval is 0.454 and 0.769). The sensitivity, specificity, PPV and NPV for various cut- offs are shown in the Table. The cutoff of ≥ 1.83 yields a sensitivity of 90% and a specificity of 26%. With the use of this cutoff, there were 1 false-negative test results and 81 false-positive test results.

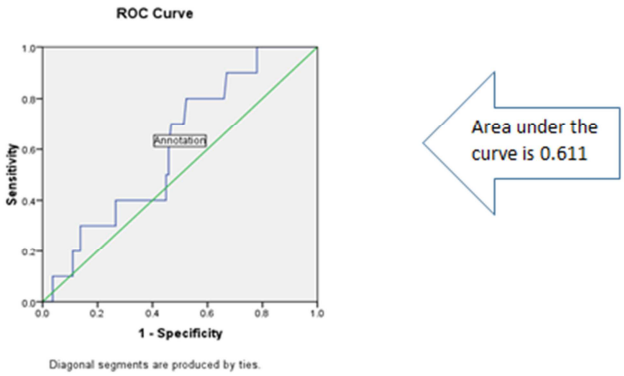

Figure 2. *ROC curve for various cutoffs of collagen-IV level as a predictor of preeclampsia.*

4. Discussion

PE is observed in 10-20% of pregnant women. It is defined as hypertension of ≥140/90 mm Hg associated with proteinuria (≥300 mg/24 h or ≥1+/random sample dipstick test), with onset after 20 weeks of gestation, persisting up to 12 weeks after delivery [10]. However, the impact of the disease is felt more severely in developing countries [24, 25]. The problem is confounded by the continued mystery of the etiology and the unpredictable nature of the disease [26]. Prediction of PE in the early stages of pregnancy can be very helpful in preventing the disorder or in decreasing its severity. It has, thus, become a major focus of research in PE. However, expected progress could not be made in this area due to the deficiency in the understanding of the pathophysiology of the disorder. Generalized endothelial dysfunction is known to be associated with preeclampsia and attempts have been made to use the biochemical markers for microvascular damage in early pregnancy to predict the development of PE in later stages. Realizing this association attention has been drawn to the biochemical markers of microvascular damage and among these, microalbuminuria (MA) got special priority.

An albumin excretion within the range of 30-300 mg/24 hrs is defined as MA [17] and its presence indicates glomerular dysfunction resulting from generalized microvascular damage. This level of albuminuria is widely accepted as evidence of established nephropathy in both diabetic and non-diabetic renal diseases.

So far, the attempt to use MA as a predictor of PE has yielded variable and given inconclusive results.

The sensitivity of prediction of PE by measuring MA in early pregnancy varied between 50% to 68%, the specificity varied between 58 to 97%, the PPV varied between 26 to 61% and the NPV varied between 87-94%. Out of 119 pregnant subjects screened 10 developed PE in later stages; thus, the prevalence is about 8.4% which is little lower than the usual values in the developing world, but seems to be reasonable is an urban setting. The present data (Table 2) shows that the group of pregnant subjects who developed PE in later stages had significantly higher values of ACR as compared to cases who did not develop PE.

In the absence of reference range of ACR in early pregnancy the ACR cut-off point for normal subjects (32 mg/g) was taken as the cut-off point in our subjects. Using this criteria 63 patients had MA, among whom 8 developed PE in later pregnancy and 55 did not develop PE.

The sensitivity of MA as a predictor of PE was found to be 80% which lies above the values reported by various authors [27, 28]. The specificity in the present study 49.54%, however, was slightly lower. The NPV in the present study was 96.42%, which corresponds to the values of 94.40% as reported by Bar et al 1996 and the PPV was 12.69% which is much lower than that reported by Das et al (1996). The substantial discrepancy between the earlier works and the present study regarding PPV may be explained by the fact that, in almost all the earlier studies, the gestational age of the subjects were higher and that increased the possibility of including already developed PE at a mild stage. Increasing the number of subjects may also increase PPV.

One of the major objective in the present study was to explore the relative merit of collagen IV in the prediction of PE. Collagen IV has recently been identified as a biomarker

of generalized vascular endothelial damage and has been claimed to be more sensitive maker than MA in diabetic nephropathy. In the present study the urinary collagen IV level in the pregnant subjects who developed PE in later stages [2.74(1.60-7.5) ng/ml] were found to be similar with those who did not develop PE [2.54(0.80-9.85) ng/ml, p=0.244]. Since there is no published data on the reference ranges of urinary collagen IV in early pregnancy we used the median value in normal subjects (2.54 ng/ml) as a cut-off point. Using these criteria 61 (88.53%) patients had high collagen (IV) and out of those 7 (11.47%) developed PE in later pregnancy. The sensitivity of urinary collagen IV in predicting PE was 70%, specificity 50.50%, PPV 11.50% and NPV 94.8%.

This is the first report where urinary collagen IV has been measured in early pregnancy and our values shows ranges almost similar to the ranges reported for normal subjects. Since Collagen IV is a relatively costly test compared to MA and since it does not give any added advantage, its use may not be advocated to predict PE in cases of pregnancy.

5. Conclusions

Generalized endothelial dysfunction is known to be associated with preeclampsia and attempts have been made to use the biochemical marker for microvascular damage in early pregnancy to predict the development of PE in later stages. Microalbuminuria (MA) a widely accepted and clinically useful biomarker for endothelial dysfunctions, but studies to use MA in predicting PE has given inconclusive results. Yagme in 1997 and Lijima in 1998 claimed that urinary collagen IV may be a useful biomarker for generalized vascular damage in diabetic nephropathy patients.

The present study was undertaken to assess the value of MA in the prediction of PE and also to compare urinary collagen IV with MA in this prediction. A total of 119 subjects (age 25±4 years, M+SD), were selected in their early pregnancy (10 to 14 weeks) and they were followed up to the term for the possible development of PE. MA was defined by albumin-creatinine ratio (ACR) above 32mg/g and high urinary collagen IV was defined by values above the cut-off point 2.54 ng/ml determined by the median value of the Controls. The data were analyzed by grouping the subjects who developed PE in later stages of pregnancy (the PE group) and those who did not develop PE in later stages (the Control group).

Out of 119 subjects 56 were primigravida and 63 were multigravida. From the total subjects 10 developed PE shows a prevalence of about 8.4%. The PE group showed a relatively higher value of ACR as compared to Control group. Although there seems to be some differences in the sensitivity, specificity, PPV and NPV of MA and high urinary collagen IV but the second in relation to the first biomarker (using proper statistical tests) showed no significant difference in predicting preeclampsia. The data suggest the following conclusions:

(1) Early pregnancy levels of microalbuminuria and urinary collagen IV can be used as predictors of PE with high negative predictive value;
(2) Urinary collagen IV has no added advantage over MA in this respect, even it is not cost-effective.

References

[1] Medicine for Africa - Medical Information Service (2008). Preeclampsia/ eclampsia. http://www.medicinemd.com/

[2] World Health Organization (2002) Global Program to Conquer preeclampsia/Eclampsia.

[3] C Dolea, C AbouZahr,"Global burden of hypertensive disorders of pregnancy in the year 2000" In: Global Burden of Diseases; 2000; World Health Organization.

[4] A Shah, B Fawole, JM M'Imunya et al. "Cesarean delivery outcomes from the WHO global survey on maternal and perinatal health in Africa", International Journal of Gynecology and Obstetrics, vol. 107 (3), pp. 191–197, 2009. [PubMed]

[5] EM McClure, S Saleem, O Pasha, RL Goldenberg, "Stillbirth in developing countries: a review of causes, risk factors and prevention strategies", Journal of Maternal-Fetal and Neonatal Medicine, vol. 22 (3), pp. 183–190, 2009. [PMC free article] [PubMed]

[6] WHO. Make every mother and child count, in The world health report 2005. Geneva, Switzerland: World Health Organization; 2005.

[7] M Warden, B Euerle, "Preeclampsia (Toxaemia of Pregnancy)", Medicine [serial online] Retrieved: 2005 May 07 Available from: URL: http://www.emedicine.com/med/topic/1905.htm [acessed on 12-11-10]

[8] C Reynold, WC Mabie, BM Sibai, AH DeCherny, L Nathan, "Hypertensive States of Pregnancy", In DeCherney AH, Nathan L (eds) Current Obstetric & Gynecologic Diagnosis & Treatment 9th ed. New York: Mc Graw-Hill; 2003: 338-353.

[9] A Conde-Agudelo, J Villar, M Lindheimer, "World Health Organization systematic review of screening tests for preeclampsia", Obstet Gynecol, vol. 104, pp. 1367–1391, 2004. [PubMed]

[10] A Conde-Agudelo, R Romero, M Lindheimer, 'Tests to predict preeclampsia", In: Lindheimer MD, Roberts JM, Cunningham FG (eds), Chesley's Hypertensive Disorders of Pregnancy, 3d ed. Elsevier; San Diego Ca: 2009. in press (May 09).

[11] RJ Levine, C Lam, C Qian, KF Yu, SE Maynard, BP Sachs, et al. "Soluble endoglin and other circulating antiangiogenic factors in preeclampsia", N Engl J Med, vol. 355, pp. 992–1005, 2006.

[12] BC Young, RJ Levine, SA Karumanchi, "Pathogenesis of preeclampsia", Annu Rev Pathol, vol. 5 pp. 173–92, 2010.

[13] F Khan, JJ Belch, M MacLeod, G Mires, "Changes in endothelial function precedes the clinical disease in women in whom preeclampsia develops", Hypertension, vol. 46, pp. 1123–8, 2005.

[14] P Verdecchia, GP Reboldi, "Hypertension and microalbuminuria: the new detrimental duo". Blood Press, vol. 13, pp. 198-211, 2004.

[15] RM Van de Wal, AA Voors, RT Gansevoort, "Urinary albumin excretion and the renin-angiotensin system in cardiovascular risk management". Expert OpinPharmacother, vol. 7, pp. 2505-20, 2006.

[16] S Chua, CW Redman, "Prognosis for preeclampsia complicated by 5 g or more of proteinuria in 24 hours", Eur J ObstetGynecolReprodBiol, vol. 43, pp. 9-12, 1992.

[17] RA North, RS Taylor, JC Schellenberg, "Evaluation of a definition of pre-eclampsia". Br J ObstetGynaecol, vol. 106, pp. 767-73, 1999.

[18] T Lijima, S Sujuki, K Sekijuka, T Hishika, M Yagme, K Jinde et al, "Follow-up study on urinary type IV collagen in patients with early stage diabetic nephropathy", J Clin Lab Anal, vol. 12, pp. 378-382, 1998.

[19] J. Clin. Lab. Anal. Vol. 11, pp. 110–116. © 1997 Wiley - Liss, Inc.

[20] MH Rodriguez, DI Masaki, J Mestman, D Kumar, R Rude, "Calcium/creatinine ratio and microalbuminuria in the prediction of pre-eclampsia". Am J Obstet Gynecol. vol. 159, pp. 1452-5, 1988.

[21] BA Fielding, DA Price, CA Houton, "Enzyme immunoassay for urinary albumin", ClinChem vol. 29 (2), pp. 355-357, 1983.

[22] M Yagme, "Significance of urinary type IV collagen in patients with nephropathy using a highly sensitive one-step sandwich enzyme immunoassay", J Clin Lab Anal, vol. 11, pp. 110-116, 1997.

[23] CP McCormick, JC Konen, ZK Shihabi, "Microtransferrinuria and microalbuminuria in the diabetic human", ClinPhysiolBiochem, vol. 8, pp. 538, 1990.

[24] G Igberase, P Ebeigbe, "Eclampsia: ten-years of experience in a rural tertiary hospital in the Niger delta, Nigeria", Journal of Obstetrics and Gynaecology, vol. 26 (5), 414–417, 2006. [PubMed]

[25] YM Adamu, HM Salihu, N Sathiakumar, GR Alexander, "Maternal mortality in Northern Nigeria: a population-based study", European Journal of Obstetrics Gynecology and Reproductive Biology, vol. 109 (2) pp. 153–159, 2003. [PubMed]

[26] FG Cunningham, KJ Leveno, SL Bloom, J Hauth, LC Gilstrap, KD Wenstrom, eds. "Hypertensive Disorders in Pregnancy", Williams Obstetrics. 22 nd ed. New York: McGraw-Hill; 2005. p. 761-808.

[27] V Das, T Bhargava, SK Das, S Pandey, "Microalbuminuria: a predictor of pregnancy-induced hypertension", Br J ObstetGynaecol, vol. 103, pp. 928-30, 1996.

[28] M Shaarawy, ME Salem, The Clinical value of microtransferrinuria and microalbuminuria in the prediction of pre-eclampsia", ClinChem Lab Med, vol. 39 (1), pp. 29-34, 2001.