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A Correlation of 8-Hour and 12-Hour with 24-Hour Urinary Protein among Pregnant Women with Preeclampsia in a Teaching Hospital in South-Western Nigeria

Akindele R.A.[1], Omopariola S.O.[1], Adeyemo A.T.[2], Afolabi B.A.[3], Awodele K.[1], Folami E.O.[4], *Adeyemo A.T.[5]

Abstract

Background and Objective: Proteinuria is one of the cardinal features of preeclampsia, which is a common and potentially severe complication of pregnancy. This study sought to determine how the quantitative measurement of urine protein from 8-hour and 12- hour samples correlate with that of a 24-hour sample in diagnosing preeclampsia.

Materials and Methods: 52 eligible pregnant women with preeclampsia were recruited between April 2017 and April 2018. For each patient, having emptied the bladder at 0 hour, urine was collected into three different containers (containers 1, 2, 3) at 8th hour, 12th hour and 24th hour ensuring that the bladder was emptied into each container at hours 8, 12 and 24. Volumes of 8 hours urine (volume in container 1), 12 hours urine (total volume in containers 1 and 2), and 24 hours urine (total volumes in containers 1, 2 and 3) were measured and 5 ml aliquot respectively obtained from each sample for colorimetric analysis of urinary protein. Data was analyzed using the EPI Info software

Results: A total of 52 patients completed the study. The mean gestational age was 33$\pm$ 2.82weeks. The mean 8-hour, 12-hour and 24-hour urinary protein values were 2.1$\pm$1.53, 2.3$\pm$1.52 and 3.1$\pm$1.89 respectively. There were significant correlations between the protein values of 8-and 12-hour urine samples with those of 24-hour urine samples

Conclusion: 8-hour and 12-hour values of urine protein correlated positively with values in 24-hour samples and may be useful for initial assessment of cases of preeclampsia for prompt interventions.

Key words: Preeclampsia, proteinuria, correlation, Nigeria

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Une corrélation de 8 heures et 12 heures avec les protéines urinaires de 24 heures parmi les femmes enceintes atteintes de pré éclampsie dans un hôpital universitaire du sud-ouest du Nigéria

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Résumé

Contexte général et objectif de l'étude : La protéinurie est l'une des caractéristiques cardinales de la pré éclampsie, qui est une complication fréquente et potentiellement grave de la grossesse. Cette étude visait à déterminer comment la mesure quantitative des protéines urinaires à partir d'échantillons de 8 heures et de 12 heures est corrélée à celle d'un échantillon de 24 heures dans le diagnostic de la pré éclampsie.

Matériels et méthode de l'étude : 52 femmes enceintes éligibles atteintes de pré éclampsie ont été recrutées entre avril 2017 et avril 2018. Pour chaque patiente, ayant vidé la vessie à 0 heure, l'urine a été collectée dans trois récipients différents (conteneurs 1, 2, 3) à la 8ème heure , 12e heure et 24e heure en s'assurant que la vessie a été vidée dans chaque récipient aux heures 8, 12 et 24. Volumes d'urines de 8 heures (volume dans le récipient 1), urines de 12 heures (volume total dans les récipients 1 et 2), et 24 heures d'urine (volumes totaux dans les conteneurs 1, 2 et 3) ont été mesurés et 5 ml d'aliquote respectivement obtenus à partir de chaque échantillon pour l'analyse colorimétrique des protéines urinaires. Les données ont été analysées à l'aide du logiciel EPI Info. Les statistiques descriptives étaient avec la distribution de fréquence, la moyenne et l'écart type, tandis que les statistiques inférentielles déployées étaient avec une analyse de corrélation et une analyse de régression simple.

Résultat de l'étude : Au total, 52 patients ont terminé l'étude. L'âge gestationnel moyen était de 33 $\pm$ 2,82 semaines. Les valeurs moyennes des protéines urinaires sur 8 heures, 12 heures et 24 heures étaient respectivement de 2,1 $\pm$ 1,53, 2,3 $\pm$ 1,52 et 3,1 $\pm$ 1,89. Il y avait des corrélations significatives entre les valeurs de protéines des échantillons d'urine de 8 et 12 heures avec celles des échantillons d'urine de 24 heures

Conclusion : les valeurs de protéines urinaires sur 8 heures et 12 heures sont corrélées positivement avec les valeurs des échantillons sur 24 heures et peuvent être utiles pour l'évaluation initiale des cas de pré éclampsie pour des interventions rapides.

Mots- clés: Pré éclampsie, protéinurie, corrélation, Nigéria

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INTRODUCTION

Hypertensive disorders of pregnancy are common major complications of pregnancy accounting for 5-10% of all pregnancies leading to significant morbidity and mortality in the fetus, the newborn infant and the mother (1,2). The preeclampsia syndrome either alone or superimposed on the chronic hypertension is the most serious hypertensive disorder in pregnancy. Preeclampsia is defined as systolic blood pressure (BP) level of 140mmHg or higher and diastolic BP of 90mmHg or higher, occurring after 20 weeks of gestation with proteinuria to the extent of >0.3gm protein in 24-hour urine specimen (3). In non-pregnant individuals, abnormal total protein excretions are typically defined as greater than 150 mg daily. In normal pregnancy, urinary protein excretion increases substantially, due to a combination of increased glomerular filtration rate and increased permeability of the glomerular basement membrane (4). Hence, total protein excretion is considered abnormal in pregnant women when it exceeds 300 mg/24 hours (3).

Proteinuria is one of the cardinal features of preeclampsia, however the severity of proteinuria is not indicative of the severity of pregnancy induced hypertension and should not be used to guide management (4,5). Also, proteinuria is part of the formal diagnostic criteria of pregnancy induced hypertension, it may still be absent. Studies have shown that 10 percent of women with clinical and/or histological manifestations of pregnancy induced hypertension have no proteinuria and 20 percent of women with eclampsia do not have significant proteinuria prior to their seizure (6,7). While renal diseases and from various sources could present with proteinuria, about 20 to 25 percent of women with chronic hypertension and diabetes develop superimposed preeclampsia (8). It is thus important for clinicians caring for pregnant women to understand how to identify proteinuria, and how to determine whether preeclampsia or renal disease (or both) is the cause.

Although proteinuria is central to the diagnosis and assessment of preeclampsia, the methods of recording its presence or extent are poorly described or standardized. The dipstick estimation of spot urine samples is the most commonly used and recorded method. However, this method is neither sensitive nor specific and studies have shown that urine dipstick for protein result correlates poorly with 24 hour urine samples for differentiating patient with no disease or severe disease (9,10). Small amounts of protein may be found in alkaline urine, urinary tract infection or contamination by vaginal discharge.

A shorter period to diagnosis would have clinical benefits such as shortened time to delivery and earlier use of antenatal glucocorticoids for fetal pulmonary maturity (11,12). Several investigators have explored other means of quantifying proteinuria in a shorter period. The protein-creatinine ratio of a single urine sample from pregnant women has been shown to correlate significantly with a 24-hour collection for patient with protein values of <1gin 24 hours. Above this level, the variation between the samples is increased. Some workers studied protein-creatinine ratios in pregnant women with preeclampsia but showed that the degree of correlation to the 24-hour sample was lower with values of>2g/24 hours (13,14). Therefore the protein-creatinine ratio is not sensitive enough to determine mild versus severe disease for patient with significant proteinuria (15,16). The gold standard for urinary protein measurement, till date, is the measurement of 24-hour urine protein (3,16). The rationale behind 24-hour collection is that in preeclamptic patients, fluctuation of protein loss in urine varies significantly over a 24-hour period and collection of less than this duration may not accurately reflect the actual amount of daily protein loss. But even with this method, there is a major drawback, as it requires too much time for collection and is cumbersome for the patient leading to poor patient compliance (9). Moreover, estimation of 24-hour urine protein may be incorrect due to improper collection, improper mixing or spillage, and it also delays the diagnosis, and hence the treatment.

There is no local study available showing the correlation of 8-hour, 12-hour and 24-hour urine protein, so this study was carried out to correlate the urinary protein levels in 8-hour, and 12-hour urine samples with 24-hour urine sample in patients with preeclampsia. Determining level of relationship (and establish correlations if any) between the 8-hour and 24-hour urine protein values in pre-eclamptic patient on the one hand, and between the 12-hour and 24-hour urine protein value in pre-eclamptic patient on the other hand would greatly assist the clinician in effective patients evaluation and management. Thus the objective of this study was to determine how the quantitative measurement of urine protein values from 8-hour and 12- hour samples correlates with that of a 24- hour sample in diagnosing preeclampsia.

MATERIALS AND METHODS

Study site: The study was conducted over a 12-month period (April 2017 to April 2018) at the ante-natal ward of UNIOSUN (formerly LAUTECH) Teaching hospital. The hospital is located in Osogbo the capital of Osun state. The teaching hospital is a tertiary health facility and it offers both specialized and general healthcare to the inhabitant of Osogbo and its environs. The prevalence of preeclampsia in this hospital is 8.3% while that of eclampsia is 2.1% (17). The hospital records an average of 1300 delivery annually and it is a referral center for other hospitals in the state and neighboring states in south-western Nigeria.

Study design: This study is a hospital-based descriptive cross-sectional study aimed at correlating the 8-hour, 12-hour with the 24-hour urinary protein in pre eclamptic women.

Study population: The study population consists of pregnant women with preeclampsia admitted to the ante-natal ward of UNIOSUN Teaching Hospital, Osogbo. Pregnant women with preeclampsia whose gestational age were >20weeks were included, while those who did not complete the 24-hour of collection because of delivery or seizure were excluded from this study.

Sample size estimation: The Leslie Fischer Formula calculated a sample size of 35 ((using a previous study (24-hour urine protein value) was 0.3 (18), and taken to 39 to account for non-response and eventually 50 for better representation. d = level of precision (desired margin of error) =0.10

Urine Collection: The patients were carefully educated about the research work and having signed an informed consent form, the patient empties the bladder initially without collecting the urine and note the time. The urine samples were subsequently collected in three separate colour labelled containers.

The first container holds the first 8-hours of urine, the second container holds the next 4-hours of urine and the third container holds the remaining 12-hour urine sample, all kept in a cool place. Total collection time was 24-hours. Each container was labelled with the patent's name, container number and collection time and date. Upon completion of 24-hour urine collection, samples were retrieved and taken to the laboratory. The sample in each container was thoroughly mixed to ensure homogeneity of the sample.

The urine volume of container #1 was measured using a graduated cylinder and recorded, a 5ml aliquot was obtained and placed in clean urine vial and label as bottle #1 and represented the 8-hour urine collection. The urine volume of container #2 was obtained in the same way after which the sample in container #2 was mixed with the content of container #1. After thorough mixing, a 5ml aliquot was obtained from the mixture placed in clean urine vial and labelled as bottle #2 and represented the 12-hour urine collection. The urine volume in container #3 was measured and then subsequently combined with the mixture of the samples from container #1 and container #2. After thorough mixing, a 5ml aliquot was obtained from the mixture, placed in a clean urine vial and labeled as bottle #3 representing the 24-hour urine collection.

The total volume for the samples was computed as follows:
8-hour urine collection: volume measured container #1.
12-hour urine collection: total volume of container #1 and container #2.
24-hour urine collection: total volume of container #1, #2 and container #3.

Analysis of the protein in each of the three aliquots was performed with colorimetric method using 5% sulfa salicylic acid. A 500microlitre aliquot of each sample was mixed with 2.5ml of 5% sulfa salicylic acid and this solution was analyzed using a spectrophotometer at 570nm wavelength. The reading was then converted to protein value using a standardized table. Samples were run in duplicate and the mean value was used for the computation for total protein. The protein value was multiplied by the total volume to obtain the total protein in a given period.

Data analysis: All results of urinary protein obtained from the laboratory were entered into Epi info version 7 software. Data was cleaned and a descriptive analysis of the data was done. Measures of central tendency and variation of variables such as age, parity, gestational age e.t.c. were also done. Pearson correlation coefficient was used to determine the strength and direction of relationship between the variables (8-hour, 12-hour and 24-hour urinary protein values). The level of significance was put at 5% (i.e. $\acute{\alpha}$= 0.05).

Ethical consideration: Ethical approval was obtained from the ethics and research committee of the institution (protocol number LTH/EC/2016/06/273). Written informed consent was obtained from each patient after explaining the purpose and objectives of the study to them and confidentiality was maintained.

RESULTS

A total of 58 patients participated in the study, and 6 of them were excluded because of delivery prior to the collection of samples. The remaining 52 patients did complete the study. The admission characteristics of participants are shown in table 1. Most of the patients (71.16%) were in the age group of 21-35 years, and the mean age (±SD) was 31.38 ± 5.80 years. The mean gestational age was 33± 2.82 weeks, while 97.6% of the subjects had gestational age ≥28 weeks. In this study preeclampsia was found more in nulliparous 46.15% and the mean parity was 1.1±1.35. Out of the 52 patient that participated in the study, 86.54% had neither previous preeclampsia nor chronic hypertension while 13.46% had either previous preeclampsia or chronic hypertension. Using 5g as the cut-off for severity, 42.86% had previous history of preeclampsia and had mild condition in index pregnancy while 57.14% had severe form in index pregnancy. Those without previous preeclampsia/ chronic hypertension were 75.56% and 24.44% with mild and severe forms in index pregnancy respectively.

The mean 8-hour, 12-hour and 24-hour urinary protein values (±SD) were 2.1±1.53, 2.3±1.52 and 3.1±1.89 respectively. There was a significant correlation between the values of 8-hour urine samples and those of 24-hour urine samples (Pearson Correlation, correlation coefficient (r) =81%, r^2=65%, P<0.000001) figure 1, also the values of 12-hour urine samples correlate significantly with that of 24-hour urine samples (r)=82%, (r^2) =67%, p<0.000001(figure 2).

With 5g as the standard severity cut-off and using 24-hour urinary protein value as a reference to which 8-hour and 12 hour is to be correlated, the receiver operating characteristic curve (ROC Curve) was used and the area under the curve for the 8-hour urinary protein was 0.912(P<0.0001) giving the 8-hour urinary protein the sensitivity of 80.00%, specificity of 91.90%, positive predictive value (PPV) of 100.00% and negative predictive value (NPV) of 75.5% and the critical value predictive of severity at 8-hour was >2.64g (figure 3) while the 12-hour urine values has area under the curve of 0.895(p<0.0001) with sensitivity of 93.33%, specificity of 73.00%, PPV of 100.00% and NPV of 78.7% and the critical value predictive of severity at 12-hour was >1.94g (figure 4). The ROC curve (Figure3)was plotted based on the critical value with the highest sensitivity and specificity for both 8-hour and 12-hour urinary protein and this accounted for the critical value of urine protein at 8-hour being greater than that of 12-hour. Otherwise, with higher critical value for 12-hour urine protein, the sensitivity and specificity will be lower and this may not be predictive of severity. Using a linear regression analysis, the 8-hour(y=1.996x+1.03) urinary protein becomes significant at 150mg while the 12-hour(y=1.213x0.7942) urinary protein becomes significant at 247mg.

DISCUSSIONS

A major problem in the diagnosis of preeclampsia is that the optimal method of establishing abnormal levels of urine protein is not thoroughly defined (4,14). The dipstick method was known to have low sensitivity and specificity for urinary protein excretion over 24 hours (19). A shorter period of urine collection to diagnose proteinuria would have clinical benefits such as shortened time of delivery and earlier use of antenatal glucocorticoids. Moreover, a more expedient intervention could decrease prenatal and maternal morbidities.

In this study, we had a small number of patients with severe preeclampsia. Such a small number might be due to the recent improvement of prenatal care. Moreover, because of the need for urgent termination of pregnancy in severe preeclampsia, there is not significant time for the 24-h urine collection.

In this study, the mean maternal age (31.38±5.8years) was comparable with other studies by Rinehart *et al*. (25 ± 6.5 years) (20), Tara *et al*. (median age 25 years) (21) and Kieler *et al*. (15). The mean gestational age (32.5±2.81weeks) in this study was also comparable with study by Rinehart et al. who observed mean age of 29 ± 4.7 weeks (20). In another study by Adelberg *et al*., mean gestational age was 33.0 ± 2.8 weeks and 30.9 ± 2.1 weeks for mild and severe preeclampsia, respectively (13). Tara *et al*. conducted a study on 26 patients in which 92.3% were in third trimester and 7.7% were in second trimester of pregnancy (21).

The results of this study indicate that the

protein values for the first 8-hour and 12-hour period do correlate with that of the 24-hour sample for patients with mild and severe proteinuria. Therefore, it might be taken as evidence to suggest that the 8-hour and 12-hour urine collection might be used to predict or diagnose mild or severe form of the disease. The level of severity in this study was based on the standard value of 5g and above in a 24hr urinary protein estimation. Using 24-hour urinary protein value as a reference to which 8-hour and 12 hour is to be correlated, the 8-hour urinary protein has sensitivity of 80.00%, specificity of 91.90%, PPV of 100.00% and NPV of 75.5% with a critical value and severity cut-off of >2.64g while the 12-hour urine values has sensitivity of 93.33%, specificity of 73%, PPV of 100% and NPV of 78.7% with a critical value and severity cut-off of >1.94g. The 8-hour urinary protein becomes significant at 150mg while the 12-hour urinary protein becomes significant at 247mg. this value is higher than the optimal cut-off point for 12-hour urine protein of 150mg documented in a review by Bartal *et al.* (22) and >165mg in a study report by Tun C *et al*. (23).

Many studies have been carried out to study the correlation of level of proteinuria during different collection periods with that of 24 hour-urine protein. Rinehart *et al*. studied the correlation of two consecutive 12-hour urine samples with that of a 24-hour urine collection in 29 patients of preeclampsia and showed sensitivity of 96%, specificity of 100%, positive predictive value of 100%, negative predictive value of 80%, and a correlation coefficient of 0.89 (20).

Keiler *et al*. compared urine albumin in spot and 12-hour urine samples with 24-hour urine collection in 30 women with preeclampsia. It was found that 12-hour collection correlated well with 24-hour collection, but the association of spot and 24-hour urine albumin was weak. So, they concluded that 24-hour urine collection can be substituted with 12-hour collection (15). This study also showed strong correlation ($p<0.000001$ figure2) between 12- and 24-hour urine samples.

Several other studies have been done for the evaluation of proteinuria in a shorter period (2, 4, 6, 8 and 12 hours), and all of them have revealed that it is possible to determine proteinuria and its severity using a shorter time of urine collections (10,11). Somanathan found significant correlation between 2-hour urine protein and 24-hour urine protein and concluded that 2-hour urine protein collection is more reliable than dipstick (11).

Amy *et al*., comparing the value of 8- and 12- hour urine protein with 24-hour, showed a meaningful correlation in patients with mild and severe proteinuria (13). Another study has shown a meaningful correlation between 4-hour and 24-hour urine protein collections in women with hypertensive disorder (14). However, a number of studies recommend more studies to confirm their own findings, and to generate exact and reliable cut-off values for predicting mild and specially severe preeclampsia (24,25).

The number of recruited patients (52 patients) in this study was about the size those of other studies. They all were inpatients and at bed rest, therefore, there was less or negligible diurnal variation in protein excretion (6). The sensitivity and cutoff values of mild preeclampsia in the present study were similar to those of Adelberg and colleagues (13). Also, the cut-off values for the diagnosis of severe preeclampsia in this study was the same with that of Adelberg *et al*.(13).

CONCLUSION

The findings of this study indicate that the 8-hour and 12-hour values of urine protein correlated positively with values of 24-hour samples. This might be used as evidence to suggest the values of total urine protein of 8-hour and/or 12-hour samples might be used for initial assessment of preeclampsia. The use of such samples for the assessment of preeclampsia helps avoid the patients' inconvenience and delay in the treatment of the disease. Based on the strong correlation of the 8-hour and 12-hour urinary protein quantification with that of 24-hour urine protein samples and the higher sensitivity of both the 8-hour and 12-hour urine samples in this study, it is recommended that the 8-hour quantification may be substituted for the 24-hour urine collection as shorter period to diagnosis would have clinical benefits such as shortened time to delivery and earlier use of antenatal glucocorticoids.

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Conflict of interest: Authors declare that they have no conflict of interest.

Author's contribution: ARA conceived the idea of the research, ARA, OSO, AK and AAT5 designed the study. ARA, OSO, AAT2, ABA, AK, FEO and AAT5 were involved in data collection as well as collection of urine specimen, ARA,

AAT2 and AAT5 were directly involved in processing of urine specimens, ARA, AAT2 and AAT5 produced initial manuscript draft, all authors provided intellectual contributions to the final manuscript. All authors read and approved the final version of the manuscript**.**

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Table 1: The admission characteristics of participants

Variables	Frequency	Percent (%)
Parity		
0	24	46.15
1	12	23.08
2	7	13.46
3	5	9.62
4	3	5.77
5	1	1.92
Total	**100**	**100.00**
Age group(Years)		
<=20	2	3.85
>20-35	37	71.16
>35	13	24.99
Total	**52**	**100.00**
Previous Preeclampsia/Eclampsia		
No	45	86.54
Yes	7	13.46
Total	**52**	**100.00**

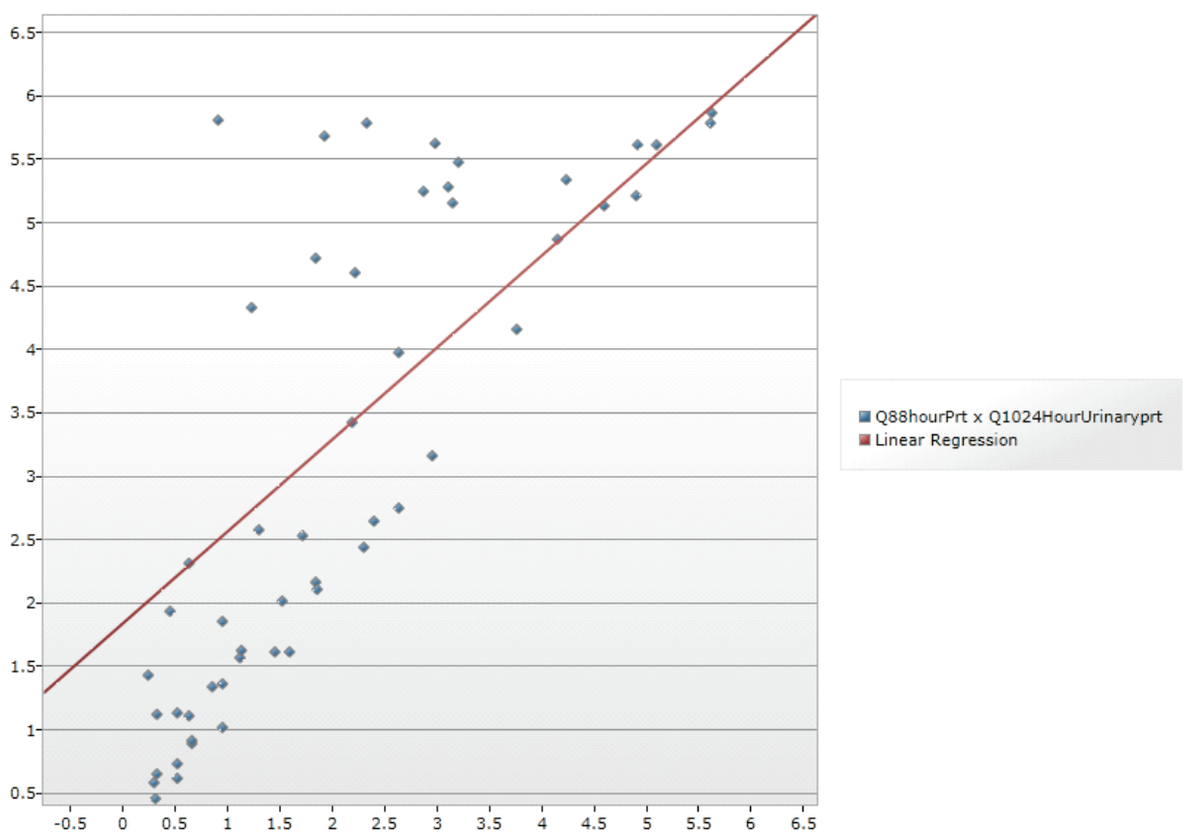

Figure 1: Scatter Diagram showing correlation of 8-hour with 24hour urinary protein values. Correlation coefficient r^2 = 0.65Coefficient of determination r = 0.81

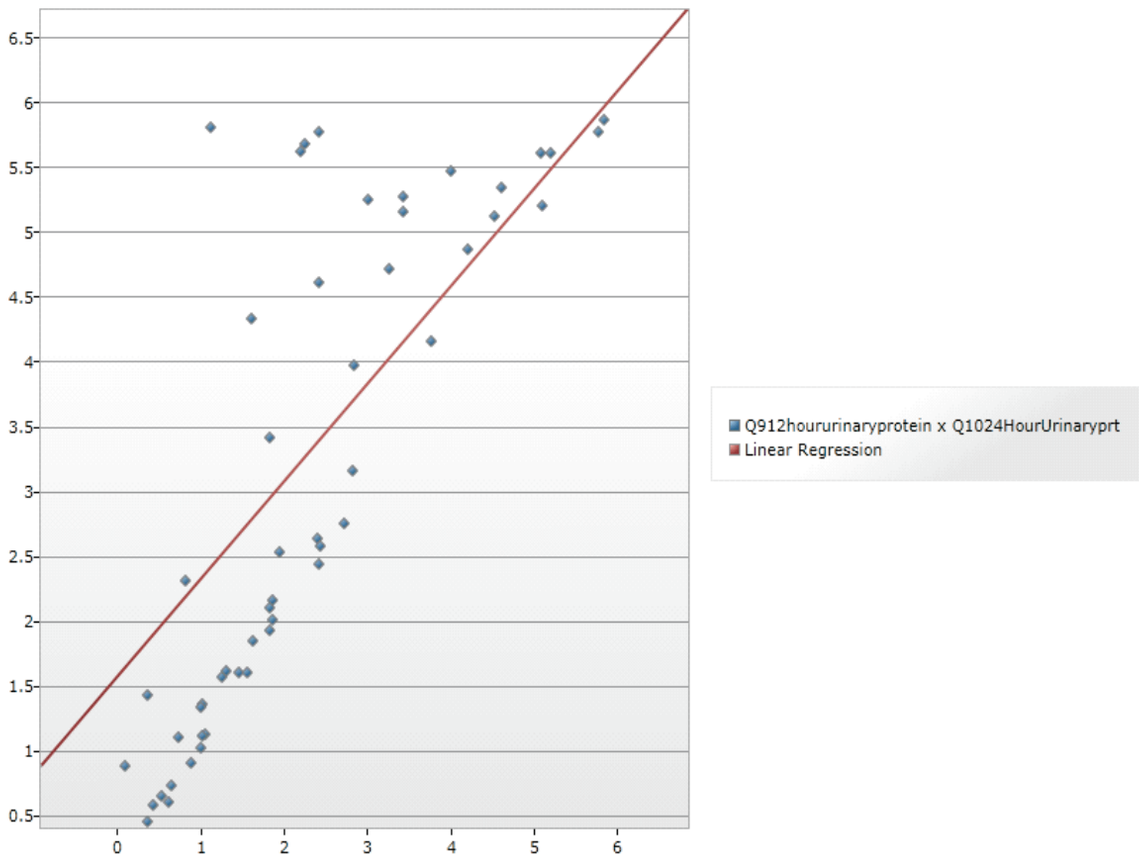

Figure 2: Scatter Diagram showing correlation of 12-hour with 24hour urinary protein values. Correlation coefficient r^2 = 0.67Coefficient of determination r = 0.82

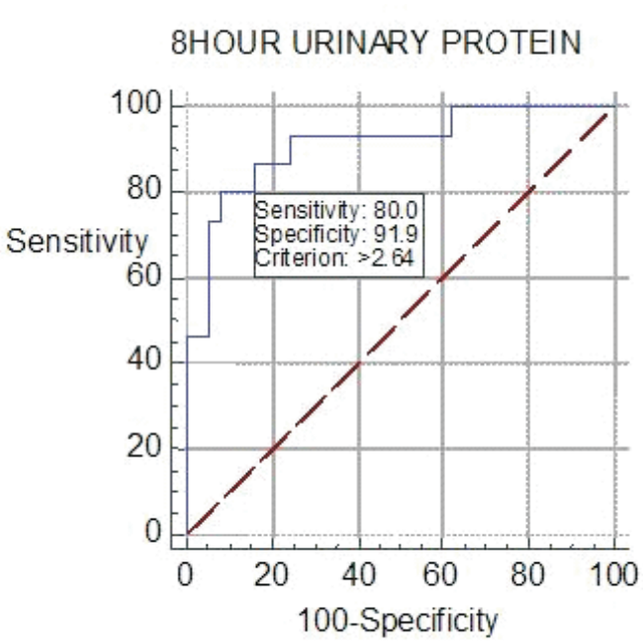

Figure 3: The ROC curve of 8-hour urine samples.

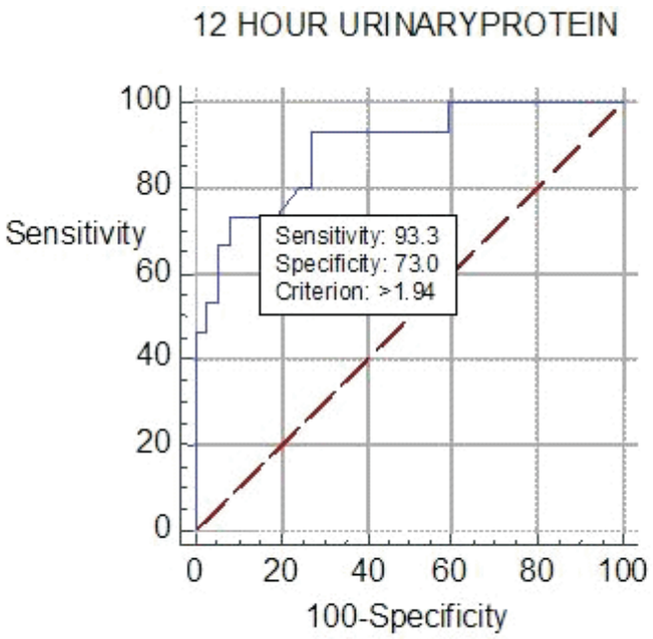

Figure 4: The ROC curve of 12-hour urine samples.