Correlation of the 4-hour, 8-hour, and 12-hour Urine Protein Values with the 24-hour Proteinuria in Hospitalized Patients with Hypertensive Disorders in Pregnancy*

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Prompt diagnosis using an accurate and quick laboratory test would promote timely intervention and result in a decrease in maternal mortalities caused by pregnancy induced hypertension. The gold standard in diagnosing proteinuria in preeclampsia, is both tedious and time-consuming resulting to delayed management or poor compliance. This cross-sectional study aimed to determine whether 4-hour, 8-hour and 12-hour urine protein values correlate with the 24-hour urine protein value in women with hypertensive disorders in pregnancy.

Pregnant patients diagnosed with hypertensive disorders in pregnancy admitted at the Obstetrics Ward and Labor Room of the Philippine General Hospital who are more than 20 weeks age of gestation were included in the study. The 24-hour urine collection was modified accordingly and subdivided into the 4-, 8-, 12- and 24-hour collection. Total volume, total protein and urine creatinine for the samples were obtained and the results were analyzed using the Pearson correlation. Only the 4- and the 8-hour samples were found to be statistically significant variables associated with the 24-hour sample. Cut-off values for the 8-hour sample were determined to be <100mg for no proteinuria and >657mg for severe proteinuria.

Key words: preeclampsia, urine protein, hypertension, pregnancy

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According to the Maternal and Neonatal Program Effort Index, over 500,000 women and girls die of pregnancy-related complications and childbirth every year, with 99 percent of cases occurring in developing countries like the Philippines. In the 2008 UNICEF report, 9 percent of these maternal mortalities are due to hypertensive complications in pregnancy, translating to 44,550 mothers dying per year as a result of elevated blood pressure and its complications, particularly preeclampsia and eclampsia.

Prompt diagnosis using an accurate and quick laboratory test would promote timely intervention and result in a decrease in maternal mortalities caused by pregnancy induced hypertension.

Hypertensive disorders of pregnancy are composed of a spectrum of disorders which involve, on at least two determinations, a systolic blood pressure of at least 140mmHg or a diastolic blood pressure of at least 90mmHg which occurs at various age of gestation, with or without the presence of proteinuria. According to the National High Blood Pressure Education Program's Working Groups on High Blood Pressure in Pregnancy (NHBEP), hypertension in pregnancy is related to one of four conditions: 1) chronic hypertension which is elevated blood pressure before 20 weeks age of gestation or persists 12 weeks postpartum; 2) preeclampsia/eclampsia, a serious systemic syndrome of elevated blood pressure occurring after 20 weeks gestational age, proteinuria and other findings like headache, abdominal pain, and abnormal laboratory results (i.e. hemolysis, elevated liver enzymes, and low platelets); 3) chronic hypertension with superimposed preeclampsia; and 4) gestational hypertension or non-proteinuric hypertension of pregnancy which arises...
at the earliest at 20 weeks age of gestation and is resolved by 12 weeks postpartum.

Preeclampsia remains a major cause of maternal and perinatal morbidity and mortality in developing countries with an incidence of 5%-6% of all deliveries. It is characterized by widespread physiologic changes, including vasospasm and activation of the coagulation system. These changes result in ischemic changes in the placenta, kidney, liver and brain, as well as a risk for bleeding complications. In the Philippine General Hospital, based on Perinatology Statistics for the past 5 years, prevalence of hypertensive disorders complicating pregnancies is higher at 12%-18% of all deliveries.

During pregnancy, proteinuria has traditionally been regarded as a hallmark of preeclampsia and an indicator of its severity. Significant proteinuria has been defined as one 24-hour urine collection with a total protein excretion of 300mg or more and a proteinuria of greater than or equal to 2 grams in 24 hours denotes a higher severity of disease. In contrast, the American College of Obstetrics and Gynecology (ACOG) recognizes 5 grams or more of protein per 24-hour urine collection as an indicator for the diagnosis of severe preeclampsia. Proteinuria in preeclampsia is brought about by glomerular damage resulting to enhanced protein permeability and decreased selectivity in tubular reabsorption of smaller proteins across the glomeruli giving rise to the presence of protein in the urine. However, given the vasospastic nature of preeclampsia, the degree of proteinuria may fluctuate widely from hour-to-hour, even in severe cases resulting to discrepancies between random protein determinations and the 24-hour urinary total protein collection. Hence, the NHBEP Working Group recommended that diagnosis be made based on the 24-hour urine collection for the total protein.

However, given the adverse complications that could be brought about the preeclampsia, prompt diagnosis made at a shorter amount of time would have clinical benefits such as shortened time to delivery and earlier use of antenatal glucocorticoids. A more expedient intervention could decrease perinatal morbidity. In addition, those women without preeclampsia would be discharged to home earlier, if a more rapid and accurate determination of proteinuria was available, resulting to lower health care cost. Patient testing may also improve if the test for proteinuria can be simplified or shortened since collecting urine over 24 hours is a cumbersome procedure for most subjects.

Hence, several studies have materialized in order to address this concern. Dipstick reagent strips are cheap, readily available, easy to administer and it detects most proteins in the urine especially albumin. However, in addition to this method being highly observer-dependent, Keiller, et al. in 2003 tested the validity of urinary albumin concentration measured by spot urine samples and concluded that since the difference between the 24-hour collection and the spot samples were too great, spot samples for albumin analysis are of poor clinical value if precise quantification of albumin excretion is required. Since urinary dipstick is a measure of protein concentration instead of its absolute value, factors such as patient’s hydration status, sample contamination with vaginal secretions and/or blood, infection, pH and specific gravity could contribute to the deviation of the result from the gold standard. A systematic review involving 6 studies measured the post-test probability of urine dipstick in predicting 24-hour urine protein excretion of 300mg to be only in the range of 53%-86% when the result was 1+ and was 23%-40% when the dipstick was negative or revealed a trace. Mayer and colleagues found that urinary dipstick of trace or negative proteinuria had a negative predictive value of 34 percent in hypertensive pregnant patients and that values of 3+ or 4+ were positively predictive of severe preeclampsia in only 36%.

Another option is the albumin: creatinine ratio, which “corrects” for dilution of concentration by taking the urinary creatinine concentration into account. The use of the ratio of protein to creatinine in the same urine specimen makes allowance for variation in the urine concentration that occurs during the day. This method, according to Ebeigbe, has been reported to have a sensitivity of 91%-93%, specificity of 88.5%-90%, positive and negative predictive values of 83%-87% and 93.9%-95%, respectively in patients with hypertensive disorders in pregnancy. However, in a systematic review it was concluded that random protein/creatinine ratio determinations are helpful primarily when they are below 130-150mg/g, in that 30mg or more proteinuria is unlikely below this threshold. But midrange protein/creatinine ratio (300mg/g) has poor sensitivity and specificity; hence it is not an ideal diagnostic test to replace the 24 hour urine collection for the diagnosis of preeclampsia.

Presently, several studies are trying to determine the potential of portions of the 24 hour urine collection in diagnosing preeclampsia. The study of
Adelberg in 2001 noted that the 8-hour urine protein results correlated with the 24 hour result for patients with mild and severe proteinuria with 84% sensitivity and 90% specificity for mild proteinuria having a cut-off of 110mg. In terms of severe proteinuria, with cut-off of 1400mg, the 8-hour urine collection result had 100% sensitivity and a specificity of 97%.

However, this study also showed that the 8-hour urine value did not correlate in cases where there was no proteinuria. The same study also showed significant correlation between the 12-hour and the 24-hour results. A similar study conducted by Sosi in 2009 involving 50 preeclamptic patients showed that values of 2-hour and 4-hour urine proteins in total volume correlated well with the 24-hour value for mild and severe disease. There was a 100% positive predictive value and 80% negative predictive value in making the diagnosis of mild preeclampsia using the 8-hour urine. Another study involving 38 in-patients who were initially diagnosed with hypertensive disorders of pregnancy showed the total protein values of the 4-hour samples positively correlated with the values of the 24-hour sample. Their laboratory examinations may then prove to be useful in the assessment of preeclampsia thereby avoiding delay in the treatment and the inconvenience of obtaining the sample.

Locally, a study by Cabalona, et al. in 1999 revealed significant correlation between the 12-hour protein value and the 24-hour urine protein value. It showed that the 12-hour protein value has a sensitivity of 86.5% and specificity of 68.2% in detecting significant proteinuria. It recommended that further studies be done to strengthen the relationship of the 12-hour protein and the 24-hour gold standard in order to establish that the 12-hour value is a good alternative to the gold standard. At present, no local study has been made to establish whether foreign data, specifically the results for the 4- and 8-hour samples, are applicable to the total population. In 2008, the OB Admitting Section of the Philippine General Hospital admitted a total of 5,928 patients, 423 of whom had preeclampsia and 211 had gestational hypertension. It would be for these patients' benefits if this local study would produce positive results.

This cross-sectional study aimed to determine if a patient's 4-, 8- and/or 12-hour urine total protein values correlate with the 24-hour value to confirm the diagnosis of preeclampsia. Specifically, this study aimed to determine which among the 4-, 8-, and 12-hour urine total protein results have the best correlation to the gold standard. It also aimed to determine the specificity and sensitivity of these parameters in diagnosing preeclampsia and to determine cut-off values in the 4-, 8-, and 12-hour urine protein result which correspond to the 300mg and 2 gram value in the 24 hour urine sample.

MATERIALS AND METHODS

Study Subjects

All admitted patients at the Obstetrics Ward of the Philippine General Hospital, initially diagnosed with hypertensive complications in pregnancy, specifically, singleton pregnancies which on at least two determinations, have a systolic blood pressure of at least 140mmHg or a diastolic blood pressure of at least 90mmHg which occurs at various ages of gestation, with or without the presence of proteinuria. However, those patients with concurrent diagnosis of urinary tract infection, diabetes mellitus, pre-existing renal disease or those with elevated creatinine levels were excluded.

The sample size was determined using a Multi-stage Size computation. After analysis of data from 31 subjects, minimal variance was already noted hence a sample size of at least 31 was noted to be sufficient.

Methodology

After obtaining the consent of patients eligible for the study, patient data forms were filled up and notes were made in the patients' chart indicating the modified manner of collecting the 24 hour-urine sample. The nurse-on-duty was informed of the patients' participation in the study.

The urine samples were collected using a foley catheter continuously draining to a urine bag to ensure standardization of urine collection. The urine bag was drained at 6:00 AM and the patients' urine samples were collected over 24 hours with the first 4 hours (6:01 AM to 10:00 AM), second 4 hours (10:01 AM to 2:00 AM) third 4 hours (2:01 PM to 6:00 PM) and remaining 12 hours (6:01 PM to 6:00 AM) collected in separate, clean 1-liter IV bottles labelled Bottle #1, Bottle #2, Bottle #3 and Bottle #4, respectively. Each container was labelled with the patient's name, bottle number and collection time and date. In cases where the urine volume per collection time exceeded the bottle provided, additional bottles were used and labelled according
to the time collected. Once the urine collection was finished, the foley catheter was removed.

Upon completion of the 24-hour urine collection, the principal investigator retrieved the samples. The sample in each bottle was thoroughly mixed with a stirring rod to ensure homogeneity of the sample. The urine volume of Bottle #1 was measured using a graduated cylinder and recorded. A 5ml aliquot was obtained and placed in a clean urine vial and labelled as Bottle #1 and represented the 4-hour urine collection. The urine volume of Bottle #2 was obtained in the same way after which the sample in Bottle #2 was mixed with the contents of Bottle #1. After thorough mixing, a 5ml aliquot was obtained from the mixture, placed in a clean urine vial and labelled as Bottle #2 and represented the 8-hour urine collection. The urine volume in Bottle #3 was then taken and the urine sample in Bottle #3 was mixed with the samples from Bottles #1 and #2. From the mixture of the 3 samples, a 5ml aliquot was obtained, placed in a clean vial and labelled Bottle #3 and represented the 12-hour urine collection. Bottle #4 was also measured for the total urine volume then subsequently combined with the mixture of the samples from Bottle #1, Bottle #2 and Bottle #3. After thorough mixing, a 5ml aliquot was obtained from the mixture, placed in a clean urine vial and labelled as Bottle #4, representing the 24-hour urine collection. The total volume for the samples was computed as follows:

- **4-hour urine collection**: volume measured in Bottle #1
- **8-hour urine collection**: total volume of Bottle #1 and Bottle #2
- **12-hour urine collection**: total volume of Bottle #1, Bottle #2 and Bottle #3
- **24-hour urine collection**: total volume of Bottle #1, Bottle #2, Bottle #3 and Bottle #4

The urine samples were then brought by the principal investigator to the Clinical Chemistry Section of the Department of Laboratories of the Philippine General Hospital for the determination of the total protein and urine creatinine.

Analysis of the protein in each aliquot was done using turbidimetric analysis. A 500 microliter aliquot of each sample was mixed with 2.5ml of 3% sulfasalicylic acid and this solution was analyzed using a spectrophotometer at 640 nm 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.

**RESULTS AND DISCUSSION**

A total of 72 eligible patients consented to take part in the study. Of these, 26 were considered dropouts from the study because either their 24-hour urine collection was improperly collected in the different bottles or there was inadvertent spillage of the urine while ongoing collection and the patients were no longer amenable to a repeat collection. Data from the remaining 46 patients were included in this study which exceeded the sample size determined using the multi-stage sample size computation.

A linear regression model was run using the data and the coefficient of determination ($r^2$) was 94% ($p<0.001$). The linear model was found to be:

$$\text{TP24} = -2.51(\text{TP4}) + 4.45(\text{TP8}) + 0(\text{TV12}) + 0(\text{TV24}) + 0.31(\text{age}) - 0.375(\text{score 3})$$

**Table 1. Statistical predictors of 24 hour total protein**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>Significance</th>
<th>Tolerance</th>
<th>VIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP4</td>
<td>-2.507</td>
<td>0.005</td>
<td>0.065</td>
<td>15.482</td>
</tr>
<tr>
<td>TP8</td>
<td>4.452</td>
<td>0.000</td>
<td>0.065</td>
<td>15.316</td>
</tr>
<tr>
<td>TV12</td>
<td>0.000</td>
<td>0.004</td>
<td>0.281</td>
<td>3.554</td>
</tr>
<tr>
<td>TV24</td>
<td>0.000</td>
<td>0.004</td>
<td>0.310</td>
<td>3.229</td>
</tr>
<tr>
<td>Age</td>
<td>0.031</td>
<td>0.054</td>
<td>0.930</td>
<td>1.075</td>
</tr>
<tr>
<td>Score 3</td>
<td>-0.375</td>
<td>0.098</td>
<td>0.923</td>
<td>1.084</td>
</tr>
</tbody>
</table>
This indicates the total protein in the first 4 and 8 hours are statistically significant variables associated with the total protein in the 24 hour collection.

Both TP4 and TP8 have a variance inflation factor (VIF) of more than 10, signifying collinearity, defined as a linear relationship between two independent variable which could result in less precise estimate of these variables’ impact on TP24 (Table 1). Collinearity is then addressed by dropping or removing one of the collinear variables from the model. Table 2 shows the analysis of the total protein for the various time frames using the Pearson correlation. The best correlation was noted with the 12-hour total protein which was 0.948, followed by the 8-hour total protein which was 0.986 then the 4-hour urine protein which was 0.866. Hence between TP4 and TP8, the variable better correlated to TP24 is maintained, simplifying the linear regression model to $TP24 = 3.055 (TP8) - 0.006$, with a coefficient of determination of 90%.

Table 2. Pearson correlation of total protein from the various time frames.

<table>
<thead>
<tr>
<th></th>
<th>TP24</th>
<th>TP4</th>
<th>TV4</th>
<th>TP8</th>
<th>TV8</th>
<th>TP12</th>
<th>TV12</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP24</td>
<td>1.000</td>
<td>0.866</td>
<td>0.158</td>
<td>0.946</td>
<td>0.205</td>
<td>0.948</td>
<td>0.091</td>
</tr>
<tr>
<td>TP4</td>
<td>0.866</td>
<td>1.000</td>
<td>0.394</td>
<td>0.961</td>
<td>0.384</td>
<td>0.932</td>
<td>0.254</td>
</tr>
<tr>
<td>TV4</td>
<td>0.158</td>
<td>0.394</td>
<td>1.000</td>
<td>0.274</td>
<td>0.877</td>
<td>0.247</td>
<td>0.751</td>
</tr>
<tr>
<td>TP8</td>
<td>0.946</td>
<td>0.961</td>
<td>0.274</td>
<td>1.000</td>
<td>0.324</td>
<td>0.988</td>
<td>0.190</td>
</tr>
<tr>
<td>TV8</td>
<td>0.205</td>
<td>0.384</td>
<td>0.877</td>
<td>0.324</td>
<td>1.000</td>
<td>0.287</td>
<td>0.804</td>
</tr>
</tbody>
</table>

With this linear regression model, the cut-off corresponding to the 300mg and 2g protein spillage in the 24 hour urine collection is determined to be 100mg and 657mg, respectively. Hence, diagnosis of mild pre-eclampsia can be made using the 8-hour urine protein spillage of 100mg, with a sensitivity of 82.1% and a specificity of 85.7% compared to the dipstick method which only has 71.8% sensitivity and 71.4% specificity in diagnosing mild preeclampsia, as seen in Table 3.

Table 3. Diagnosis of mild preeclampsia.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP4</td>
<td>100%</td>
<td>65.3%</td>
<td>84.8%</td>
</tr>
<tr>
<td>TP8</td>
<td>82.1%</td>
<td>85.7%</td>
<td>82.6%</td>
</tr>
<tr>
<td>Stat Albumin</td>
<td>71.8%</td>
<td>71.4%</td>
<td>71.7%</td>
</tr>
</tbody>
</table>

On the other hand, severe preeclampsia may be diagnosed with a protein spillage of 657mg in an 8-hour urine collection, with 100% sensitivity and 88.9% specificity. Although the dipstick technique also provides 100% sensitivity in diagnosing severe preeclampsia, its specificity is significantly decreased at 63.9% as reflected in Table 4. This may result in increased number of patients receiving unwarranted treatment for severe preeclampsia such as administration of magnesium sulphate, if stat albumin would be the basis of the diagnosis. In addition, the coefficient of determination of the dipstick method is only 48% compared to that of the 8-hour collection which is 90% signifying that the variance of the total protein in the 24 hour collection can be better explained by the 8-hour collection compared to the dipstick method.

Table 4. Diagnosis of severe preeclampsia.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP4</td>
<td>90%</td>
<td>91.7%</td>
<td>89.1%</td>
</tr>
<tr>
<td>TP8</td>
<td>100%</td>
<td>88.9%</td>
<td>91.3%</td>
</tr>
<tr>
<td>Stat Albumin</td>
<td>100%</td>
<td>63.9%</td>
<td>71.7%</td>
</tr>
</tbody>
</table>

**SUMMARY AND CONCLUSION**

The 4-, 8- and 12-hour urine protein sample correlates well with the gold standard. However, only the 4- and the 8-hour samples were found to be statistically significant variables associated with the 24 hour sample. Cut-off values for the 8-hour sample were determined to be <100mg for no proteinuria and >657mg for severe proteinuria.

With these, it may be said that 8-hour collection is an acceptable alternative to the 24-hour gold standard resulting to more rapid diagnosis and a more accurate one, compared to the dipstick method resulting to shortened time to delivery, earlier use of antenatal glucocorticoids, and more expedient intervention leading to decreased perinatal morbidity. Also, those women without preeclampsia would be discharged to home earlier resulting to lower health care cost and since the 8-hour collection is shorter than the cumbersome gold standard then there might be improved patient compliance with the 8-hour urine collection.
REFERENCES