DETERMINATION OF COVERAGE INTERVAL OF MICROPROTEIN IN URINE IN PREGNANT LADIES BY THE PYROGALLOL-RED METHOD IN PATIENTS ATTENDING A TERTIARY CARE HOSPITAL IN EASTERN INDIA

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ABSTRACT

BACKGROUND

Pre-eclampsia characterised by hypertension and proteinuria in pregnancy is a major cause of maternal morbidity worldwide. So it is important to quantify the protein excretion in urine during pregnancy to help clinicians to predict pre-eclampsia in vulnerable patients. Traditionally, the estimation of 24 hours protein excretion in urine has been used for this purpose which is a cumbersome process. The dipstick method, though an easier one, does not yield satisfactory results. The pyrogallol-red method is perhaps a more acceptable method in this regard. It has also been noted that there exists almost no relevant data on pregnancy proteinuria in this part of the country. In light of this background, we embarked on this study to determine the coverage interval of microprotein in urine by the pyrogallol-red method in pregnant patients attending a tertiary care hospital in Eastern India.

MATERIALS AND METHODS

This cross-sectional study was carried out on 71 pregnant ladies attending the antenatal clinic in the Gynaecology and Obstetrics Department at College of Medicine and Sagore Dutta Hospital, a tertiary care hospital in Kolkata. The protein in fresh, clean voided urine of pregnant patients was estimated by the pyrogallol-red method on ERBA Chem 5x semi-auto analyser. The statistical data thus obtained was analysed by IBM SPSS version 16.

RESULTS

It may not be always feasible to collect 120 samples to establish a reference interval as required by the IFCC. For this reason, the term coverage interval is preferred over reference interval by IUPAC. The coverage interval of pregnancy proteinuria was calculated to be 23.99—29.64 mg/dL with a confidence of 0.90 and uncertainty of 0.049.

CONCLUSION

In this study, we conclude that the coverage interval of urinary microprotein for pregnancy proteinuria is 23.99—29.64 mg/dL as determined by the pyrogallol-red method.

KEYWORDS

Coverage Interval, Pregnancy Proteinuria, Pyrogallol-red Method, Uncertainty.

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BACKGROUND

Pregnancy pre-eclampsia is a major cause of maternal morbidity complicating up to 2-8% of pregnancies worldwide.^[1,2] It is a multisystem disorder characterised by hypertension and proteinuria occurring after 20 weeks of pregnancy in previously normotensive women. It is important for clinicians caring for pregnant women to attempt to quantify proteinuria in pregnancy, with the aim of making a diagnosis as well as to anticipate the foetal and maternal outcome. Usually, any protein excretion greater than 150 mg

daily is considered to be abnormal.^[3] But in pregnant women, the protein excretion increases to about 300 mg per day due to physiological changes.^[4]

Traditionally, the gold standard for quantification of proteinuria has been the estimation of 24 hours excretion of protein in urine which has a cumbersome process of collection and is often inconvenient for ambulatory patients.^[5] The dipstick method requires a spot urine sample and is a rapid, user-friendly, inexpensive and widely used method for estimation of proteinuria with the purpose of detection of pre-eclampsia during antenatal visits.^[6] But studies have shown poor accuracy, low sensitivity and specificity associated with the dipstick method.^[7-9]

There is a paucity of data related to the coverage interval of microprotein in urine in pregnant ladies in this part of the country. In this study, we attempted to determine the coverage interval of microprotein in urine of pregnant ladies attending the antenatal outdoor in this hospital by the pyrogallol-red method. We embarked on this study with the idea that this method of estimation of the microprotein may be used as a screening method for detection of proteinuria in

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pregnancy and help in the prediction of pre-eclampsia in vulnerable patients particularly in resource poor set-ups.

MATERIALS AND METHODS

This cross-sectional study was carried out on 71 pregnant ladies attending the antenatal clinic in the Gynaecology and Obstetrics Department at College of Medicine and Sagore Dutta Hospital, a tertiary care hospital in Kolkata. All antenatal patients who gave informed consent for the study were included. Pregnant mothers suffering from diabetes, primary renal disease and other medical disorders complicating pregnancy like SLE, heart disease, hypothyroidism, Cushing's, etc. were excluded from the study. Antenatal mothers with multiple pregnancies were also excluded. Fresh, clean voided, midstream urine specimen was collected from the patients for testing for proteinuria. The estimation of protein in the spot urine thus collected was done by the Microprotein kit (pyrogallol-red method) supplied by Coral Clinical Systems on ERBA Chem 5x semi-auto analyser. In an acidic medium, proteins combine with Pyrogallol-red and molybdate to form a blue purple coloured complex. The intensity of the colour of this complex as measured at 600 nm is directly proportional to the amount of proteins present in the sample. This method could be easily adapted to automation as the dye present in the reagent does not stain the walls of the cuvette and thus do not interfere with the measurements.

The statistical data thus compiled was analysed statistically by the IBM SPSS version 16. After calculation of the standard deviation, the data was subjected to tests of normality of Kolmogorov-Smirnov. As per the results obtained, the coverage interval was calculated by the software and reported with the help of a table as described by IUPAC.

RESULTS

The results obtained after statistical analysis are described below.

Number of Patients	48
Mean of All Values	27.82 mg/dL
Median of All Values	21.67 mg/dL
Mode (only smallest value is shown)	10.73 mg/dL
Standard Deviation	18.02
Standard Error of Mean	2.71
Test for Normal Distribution	
Kolmogorov-Smirnov test	$P = 0.047$
(The Distribution is not in Normal Distribution as $P < 0.05$).	
Non-parametric coverage interval	23.99-29.64 mg/dL

Table 1. Descriptive Statistics of the Urinary Microprotein from Pregnant Ladies in Second Trimester

Source	SS	df	MS	
Between Treatments	117.0361	2	58.5181	$F = 0.15259$
Within Treatments	25694.4364	67	383.4991	
Total	25811.4725	69		

Table 2. Comparison of the Statistics Of Urinary Microprotein among the Patients belonging to the Three Trimesters (i.e. patients of first Trimester, Patients of Second Trimester and those of Third Trimester) by ANOVA

The f-ratio value is 0.15259. The p-value is 0.858779. The result is not significant at $p < 0.05$.

DISCUSSION

The renal physiology undergoes tremendous changes very early during pregnancy. The size of the kidney increases by 1.5 cm.^[10] With the increase in renal plasma flow, the glomerular filtration rate starts rising and may reach 25% higher as early as the end of the second week of conception. It escalates to 50% by the beginning of the second trimester. The pregnancy-induced haemodilution leads to a decrease in plasma protein concentration with a consequent decrease in the plasma oncotic pressure within the glomerulus. The renal plasma flow which increases by as much as 80% at the end of the second trimester may also contribute to the elevation of GFR in pregnancy.^[1] This increased GFR coupled with increased permeability of the basement membrane ultimately leads to increased excretion of protein in urine during pregnancy. This haemodilution and hypervolaemia of gestation eventually reverses by the second week postpartum.^[11]

As per IFCC, collection and analysis of at least 120 samples is mandatory for establishment of reference interval which may not be always economically viable under strict inclusion and exclusion criteria. This is particularly relevant in the establishment of reference values for those parameters in which the reference method is also costly (e.g. estimation of anti-epileptic drugs, toxic metals, antioxidants, etc.). The distribution of most biological parameters are not Gaussian. Recently, the term 'coverage interval' is preferred over the term 'reference interval' by the International Union of Physical and Applied Chemistry (IUPAC) as well as other leading expert bodies like the International Organisation for Standardisation (ISO). The term coverage interval enjoys a number of advantages. The coverage interval envisages the probability of distribution of the values in the reference population with a specified degree of confidence. Additionally, the degree of uncertainty associated with the value is statistically well-defined.^[12] In other words, the term coverage interval is somewhat similar to prediction interval. This uncertainty (δ), which is a measure of the authenticity of the calculated coverage interval, is independent of the standard deviation. In fact, it is influenced by the level of expectation (β), level of confidence (γ) and the number of values (n). For this purpose, we undertook to establish the 0.95 coverage interval of proteinuria in pregnancy (level of expectation or β) with a confidence of 0.90, (level of confidence, γ) with a statistically defined level of uncertainty (δ).

When the sample size for the calculation of the coverage interval is small, it is not possible to define a 95% confidence interval. In such cases, 90% confidence interval may be calculated as per the recommendations in the Table 1 of the IUPAC document.^[12] Therefore, a fewer number of reference values can be used to estimate a clinically useful interval which is also endowed with defined uncertainty and precision. The coverage interval, similar to the reference interval can be calculated both parametrically as well as non-parametrically. In clinical medicine, any value outside the coverage interval indicates that the individual may be at a greater health risk. Coverage intervals are extremely useful for calculation of toxic doses (the upper limits) of drugs and poison. Smaller coverage uncertainty provides with a higher precision in the estimation of the coverage interval.

Though the urinary dipstick method is simple and inexpensive to carry out, its major drawback is the high number of false positives, inaccuracy and decreased sensitivity. The term 'trace proteinuria' obtained in the routine dipstick screening method as defined by "+1" is widely accepted as a marker for pregnancy proteinuria. But this result may be unreliable from the clinical point of view as it largely depends on the maternal hydration status. False positives are also encountered with haematuria, alkaline pH, interference by detergents, drugs, high specific gravity, etc.^[13,14] A large number of false positives would eventually drain the available clinical resources for the management of pre-eclampsia in the name of prevention. In order to avoid these drawbacks, we went ahead with the pyrogallol-red method. Originally described by Fujita,^[15] the pyrogallol-red method is one of the few colorimetric methods whose reagents do not adsorb to the walls of the cuvettes and so do not interfere with the measurements. For this reason, this method could be adapted to automated analysers.^[16]

The reaction is complete within 5 minutes and the absorbance of the reaction mixture remains unchanged at 25°C–37°C. So, this method may be easily carried out at peripheral health care centres which have no access to air conditioning as prevalent in this country. The method enjoys a high linearity, almost up to 4000 mg/dL and has a high sensitivity procedure as well. This method has adequate reproducibility and recovery studies necessary for clinical use. In this study, we used a semi-auto analyser which is relatively inexpensive and may be available in resource poor set-ups like block primary health centres.

In our study, the coverage interval of urinary microprotein in the second trimester is 23.99–29.64 mg/dL and the mean value of urinary microprotein in the second trimester was 27.82 mg/dL. Comparison of the mean of urinary microprotein in all the three trimesters by ANOVA (vide Table 2), showed that there is no statistically significant difference in the mean values of urinary microprotein in the second trimester compared to the first and third. So, for all practical purposes, the coverage interval of urinary microprotein in the second trimester may be applied to the antenatal mothers in the first and third trimesters as well. Usually, a higher number of antenatal mothers seek medical advice in the second trimester (first contact) compared to the first trimester in this part of the country.

Keeping all this in mind, the 0.95 coverage interval of urinary microprotein in pregnancy, with a confidence of 0.90 is calculated to be 23.99–29.64 mg/dL with an uncertainty of 0.049 as deduced from the table recommended by the IUPAC. In other words, 'the interval [23.99–29.64] will on an average cover 95% of the distribution of all the values for pregnancy proteinuria such that the probability is at least 0.90 that the interval covers between 0.95-0.049 and 0.95+0.049 of the distribution.

CONCLUSION

In this study, we tried to estimate the coverage interval of urinary microprotein in pregnant women attending a tertiary care hospital in Eastern India by the pyrogallol-red method. This is a simple method with adequate linearity and reproducibility which can be easily adapted to peripheral health care establishments with limited infrastructure in

rural areas in this country. The IUPAC, ISO and other leading bodies have advocated the usage of the term coverage interval instead of reference interval. The coverage interval is usually declared with the level of confidence, level of expectation, and a defined degree of uncertainty, all of which are taken into account in its calculation in accordance with the recommendations of IUPAC. In this study, we conclude that the coverage interval of urinary microprotein for pregnancy proteinuria is 23.99–29.64 mg/dL in the population attending a tertiary care hospital in Eastern India as determined by the pyrogallol-red method.

REFERENCES

- [1] Cornelis T, Odutayo A, Keunen J, et al. The kidney in normal pregnancy and pre-eclampsia. *Seminars in Nephrology* 2011;31(1):4-14.
- [2] Khan KS, Wojdyla D, Say L, et al. WHO analysis of causes of maternal death: a systematic review. *Lancet* 2006;367(9516):1066-74.
- [3] Roberts M, Lindheimer MD, Davison JM. Altered glomerular permselectivity to neutral dextrans and heteroporous membrane modeling in human pregnancy. *Am J Physiol* 1996;270(2 Pt 2):F338-F43.
- [4] Higby K, Suiter CR, Phelps JY, et al. Normal values of urinary albumin and total protein excretion during pregnancy. *Am J Obstet Gynecol* 1994;171(4):984-9.
- [5] Price CP, Newall RG, Boyd JC. Use of protein: creatinine ratio measurements on random urine samples for prediction of significant proteinuria: a systematic review. *Clinical Chemistry* 2005;51(9):1577-86.
- [6] Waugh JJ, Clark TJ, Divakaran TG, et al. Accuracy of urinalysis dipstick techniques in predicting significant proteinuria in pregnancy. *Obstet Gynecol* 2004;103(4):769-77.
- [7] Amin SA, Illipilla S, Hebbar S, et al. Quantifying proteinuria in hypertensive disorders of pregnancy. *International Journal of Hypertension* Article ID 941408, 2014;2014: p. 10.
- [8] Waugh JJS, Bell SC, Kilby MD, et al. Optimal bedside urinalysis for the detection of proteinuria in hypertensive pregnancy: a study of diagnostic accuracy. *BJOG* 2005;112(4):412-7.
- [9] Dwyer B, Gorman M, Carroll IR, et al. Urinalysis vs urine protein-creatinine ratio to predict significant proteinuria in pregnancy. *Journal of Perinatology* 2008;28(7):461-7.
- [10] Studd J. The origin and effects of proteinuria in pregnancy. *BJOG* 1973;80(10):872-83.
- [11] Hladunewich MA, Lafayette RA, Derby GC, et al. The dynamics of glomerular filtration in the puerperium. *American Journal of Physiology Renal Physiology* 2004;286(3):F496-503.
- [12] Poulsen OM, Holst E, Christensen JM. Calculation and application of coverage intervals for biological reference values (Technical Report) *Pure & Appl Chem* 1997;69(7):1601-11.
- [13] Waugh J, Bell SC, Kilby M, et al. Effect of concentration and biochemical assay on the accuracy of urine dipsticks in hypertensive pregnancies. *Hypertension in Pregnancy* 2001;20(2):205-17.

- [14] Phelan LK, Brown MA, Davis GK, et al. A prospective study of the impact of automated dipstick urinalysis on the diagnosis of pre-eclampsia. *Hypertension in Pregnancy* 2004;23(2):135-42.
- [15] Fujita Y, Mori I, Kitano S. Colour reaction between pyrogallol-red molybdenum (VI) complex and protein. *Bunseki Kagaku* 1983;32:E379-86.
- [16] Watanabe, Kamei S, Ohkubo A, et al. Urinary protein as measured with a pyrogallol-red-molybdate complex, manually and in a Hitachi 726 automated analyzer. *Clin Chem* 1986;32(8):1551-4.