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COMPARISON OF CONGO RED STAINING KIT VERSUS UROCOLOR TEST TO DETECT PROTEINURIA IN PATIENTS OF PREECLAMPSIA

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Abstract:

Objective: *To compare the Congo Red staining kit with the urocolor test to evaluate the proteinuria in patients with preeclampsia.*

Study Design: *A Cross sectional Study.*

Place and duration: *In the Obstetrics and Gynecology department of Divisional Headquarter Teaching Hospital, Mohtarma Benazir Bhutto Shaheed Medical College, Mirpur AJK and Red Crescent Medical and Dental College Teaching Hospital, Lahore for one year duration from Feb 2018 to January 2019.*

Methods: *A prospective, cross-sectional study including preeclampsia-diagnosed pregnant women and prenatal women with preeclampsia diagnosed clinically. A single 24-hour urine sample was analyzed with Congo Red to determine the presence of proteinuria among all patients, the qualitative results of urine dipstick for proteinuria and the determination of protein in the 24-hour collected urine is regarded as the gold standard. Differences between nominal conditions were determined by Mann-Whitney U test.*

Results: *A total of 50 puerperal and pregnant women were selected for the study. In the statistical comparison between 2 and 3, 2 and 4, 3 to 4, the positive Congo Red in the fields reached a significant variation (p 40.001) in relation to the average proteinuria values for each. In contrast, there was no difference in the number of positive crosses with the urocolor test.*

Conclusion: *Congo red has a high selectivity for beta folded proteins in preeclampsia and is a promising high-value test for the clinician.*

Key Words: Congo red, proteinuria, urocolor test, pregnancy.

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INTRODUCTION:

During pregnancy, protein excretion increases due to a decrease in the reabsorption of the low molecular weight proteins (LMW) filtered by the glomerulus in the proximal tubule. It is important to note that even healthy pregnant women can record> 300 mg / dL proteinuria. The determination of the amount of proteinuria in a 24-hour urine sample is mandatory in the comprehensive assessment of women with hypertensive disorder during pregnancy and in some cases, it is necessary to determine the diagnosis of preeclampsia and severity[1]. The National Blood Pressure Training Program (NHBPEP) study group identifies significant proteinuria as a protein excretion of> 300 mg in a 24-hour urine sample. The same study group also indicates that it implies a greater severity of proteinuria disease of 2 g / day, and this figure is determined as a criterion for defining severe preeclampsia[2]. In contrast, the American College of Obstetrics and Gynecology (ACOG) produces 5 g / day proteinuria for the diagnosis of severe preeclampsia[3]. The quantification of 24-hour urine collection proteins is considered the gold standard for determining the presence of significant proteinuria in pregnancy. However, urine collection every 24 hours is uncomfortable for the patient, time consuming and subject to errors due to insufficient collection, delaying the diagnosis for at least 24 hours, and less than half of the women are reported. In hospitalized patients diagnosed with preeclampsia there is a 24-hour urine collection[4]. Due to the disadvantage of collecting 24-hour urine, other options for the diagnosis of significant proteinuria during pregnancy have been proposed. These other tests are fast and reliable, such as the use of labstix or dipstick, proteinuria measurement at short urine collection times, and protein / urine creatinine ratio[5]. The three-dimensional conformation of the protein is the result of dispersed polypeptides that pass through a molecular folding process in which various models are proposed[6]. False folded proteins produce amorphous aggregates, a phenomenon determined by the melting temperature (Tm). Some diseases of false protein folding include Alzheimer's, Parkinson's and some neurodegenerative diseases[7]. Interestingly, preeclampsia is characterized by an increase in excretion of misfolded protein drawn by a Congo Red called Diazo[8]. Using a non-biased mass spectrometric proteomic profile approach, Buhimschi group IA et al., Demonstrates a characteristic set of proteomic biomarkers consisting of non-randomized SERPINA1 proteoforms in urine of women with severe preeclampsia and albumin[9]. The SERPINA1 fragments tend to folding and accumulating in supramolecular structures, thus showing congophilia, a characteristic of false protein folding. The aim of this study was to compare the Congo Red staining kit with an urocolor test to determine proteinuria in patients with preeclampsia and to find a more specific option that correlated well with the 24-hour urine collection test[10].

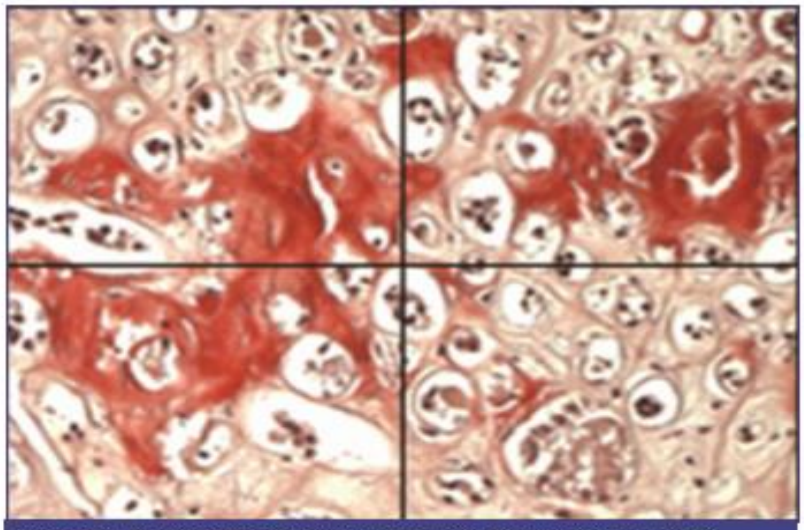
MATERIALS AND METHODS:

This prospective, cross-sectional study was held in the Obstetrics and Gynecology department of Divisional Headquarter Teaching Hospital, Mohtarma Benazir Bhutto Shaheed Medical College, Mirpur AJK and Red Crescent Medical and Dental College Teaching Hospital, Lahore for one year duration from Feb 2018 to January 2019. The sample was generated by a probabilistic sampling, based on the available Congo Red tests. The study included 50 pregnant women who presented the clinical criteria of preeclampsia, who were between 20 and <41 weeks of gestation, or immediately in the puerperium. The diagnosis of preeclampsia was consistent with ACOG recommendations. Women with a history of preeclampsia, urinary tract infection (UTI), kidney disease or pregnancy hypertension were excluded from the study. Patients with missing medical records were excluded from the final analysis. This study was approved by the Ethics Committee. Written informed consent was obtained from all patients who participated in the study.

General information: The main source of information was collected through examination of medical records and consideration of: age; education level; factors associated with pregnancy hypertensive disorder; antenatal care; the type of birth of the baby; Main indication for cesarean section and maternal and perinatal outcomes.

The Congo red Highman method was adjusted using a semi-quantitative kit from Congo Red. The Congo red staining principle is based on the formation of a hydrogen bridge with the carbohydrate component of the substrate. Congo red is an anionic dye and can then accumulate on amyloid fibrils that exhibit a noticeable dichroism under polarized light. The tissue stained with Congo red appears orange-red under the light microscope sent; under polarized light; However, amyloid deposits are shown as bright green images with double refraction on a dark background. The staining process took an average of 12 minutes by the Clinical Laboratory Service. Reading was done by two chemical pharmacologist and pathologist. To provide a report based on positive quadrants (fields), each slide is divided into four visual areas during microscopic imaging. In the Highman method, staining was carried out by separation of an alcohol solution (in this case 2%

isopropyl alcohol was used) and then with an alkaline solution (KOH). In this process, urine sediment was used as an example for study, and therefore the process steps were minimized. The sample was collected after 3 minutes of centrifugation and then fixed with heat. The next step was to paint in Congo Red solution, then rinse and dry. Interpretation, presence and distribution of microscopic field of view (quadrant) estimated [Table / Figure-1].



[Table/Fig-1]: Exemplification of the microscopic slide division in quadrants.

The center (amyloid) was reported as a positive result to green blue meteorites with pink-red pigmentation and green environmental metacromas when polarized light was used. At the same time, two measurements with a 24-hour urine collection and urocolor 11 reactive strips were performed in the first 6 hours using a chromatic scale.

The normality hypothesis was tested using the Kolmogorov-Smirnov test. Differences between nominal conditions were evaluated by Mann-Whitney U test. The intra and inter-interpersonal Kappa index was calculated for the identification of positive congestion. In all cases, p anlamlı0.05 was considered statistically significant. All tests were performed in SPSS version 23.0 statistics program.

RESULTS:

Considering 436 hospital admissions (0.88 applications per day) in the Intensive Care Unit (OICU) during the study, preeclampsia represented a total of 223 cases (51.14%) (0.44 admissions). day). In all of this population, only 50 women were elected. The mean age was 27.4 ± 7.2 years, mean hospital stay was 3.6 ± 1.5 days and there were three organic dysfunction. [Table / Figure 2] shows the general characteristics of the population studied.

Variable	Value
Age (years)	27.4±7.2
Gestational age (weeks)	33.96±2.5
Mean arterial pressure (mmHg)	111.6±24.3
Proteinuria (mg/dL)	3076.2±1677.6
Stay in the obstetrical ICU (days)	3.6±1.5

[Table/Fig-2]: General characteristics of the patients.
ICU: Intensive care unit

Age range (Years)	n (Percentage)
15-20	9 (18%)
21-25	12 (24%)
26-30	14 (28%)
31-35	6 (12%)
36-40	7 (14%)
≥40 years	2 (4%)
Total	50 (100%)

[Table/Fig-3]: Distribution of patients per age range.

With regard to obstetric data, 1 (28%) was the primary conductor and the mean number of pregnancies was 2.72. Women 's obstetric gestational age was 33.96 weeks (early preeclampsia) and 45 (90%) had risk factors. [Table / Figure 3] shows the distribution by age. The analysis of organ dysfunction is based on the classification of the Sequential Evaluation of Organ Failure (SOFA) and major organ dysfunctions [Table / Figure 4]. While on discharged,> 96% of the patients experienced improvement in all dysfunctions. 26 of the women (52%) had fetal

involvement. The fetal results are described in [Table / Figure-5].

Organ failure	n (Percentage)
Renal	50 (100%)
Haematological	31 (62%)
Hepatic	29 (58%)
Cardiovascular	28 (56%)
Neurological	13 (26%)
Respiratory dysfunction	7 (14%)

[Table/Fig-4]: Main organ dysfunctions.

Outcome	Unit
APGAR score at 5 minutes (mean±SD)	7.4±1.8
Fetal weight (gm) (mean±SD)	1951.9±742.5
Intrauterine growth restriction (cases)	12 (24%)
Cesarean section (cases)	44 (88%)

[Table/Fig-5]: Fetal outcomes.
SD: Standard deviation

Kongofilia was present in 12 areas (24%), in three areas, 34% (n = 17) and four areas (21%) (42%). Kappa index between observers 0.87 (expected, 0.5) and inter-observer Kappa index 0.740 (Standard error (SE): 0.067; 95% CI (95% CI)), 0608-0872. While protein determination in 4 (8%) was <1000 mg, in 10 (20%) 1.001-2.000, in 12 (24%) 2.001-3.000 mg, in 15 (30%) nephrotic range 3.001 to 4.000 4 (8%) showed between 4.001 and 5,000 mg and more than 5,000 mg, 5 (10%).

Congo red fields	N	Mean	Standard deviation	Error	95% Confidence interval for the mean		Minimum	Maximum
					Lower limit	Upper limit		
2	12	1366.4	375.0	108.2	1128.0	1604.7	934.2	1892.0
3	17	2664.5	471.0	114.2	2422.3	2906.7	1490.0	3349.7
4	21	4386.7	1714.3	374.0	3606.3	5167.0	2763.1	9967.1
Total	50	3076.2	1677.6	237.2	2599.5	3553.0	934.2	9967.1

[Table/Fig-6]: Proteinuria values per number of congo red positive fields.

Displaying this information [Table / Figure 6] shows the mean values of proteinuria according to the number of congo positive red areas and [Table / Figure 7], although 100% of the cases were positive for the congo red test, although none of the urocolor ribbon 11 was positive in the nephrotic range despite proteinuria symptoms.

Congo red positive fields	Urocolor 11 strip positive crosses				Total
	None	One	Two	Three	
2	6	6	0	0	12
3	9	5	2	1	17
4	6	12	3	0	21
Total	21	23	5	1	50

[Table/Fig-7]: Number of urocolor positive crosses per congo red positive fields.

Groups of comparison per congo red positive fields	Mann-Whitney U-test	W of Wilcoxon	Z	Sig.
2 vs. 3	4	82	-4.34	≤0.001
2 vs. 4	0.0	78	-4.715	≤0.001
3 vs. 4	27	180	-4.448	≤0.001

[Table/Fig-9]: Comparison of proteinuria per congo red positive fields.
Sig: Significance

Kolmogorov-Smirnov normality test was used and a non-Gaussian dispersion for proteinuria was shown by the positive Congo Red identification group. In the light of the results, Mann-Whitney U test was used to compare proteinuria values [Table 9].

On the other hand, proteinuria average values according to the number of crosses in the urocolor strip are shown in [Table / Figure 10].

Number of crosses	N	Mean	Standard deviation	Error	95% Confidence interval for the mean		Minimum	Maximum
					Lower limit	Upper limit		
none	21	2866.2	1595.8	348.2	2139.8	3592.6	958.4	6540.0
1	23	3251.2	1960.7	408.8	2403.3	4099.1	934.2	9967.1
2	5	3178.3	414.5	185.3	2663.6	3693.0	2782.0	3819.0
3	1	2952.4	.	.	.	.	2952.4	2952.4
Total	50	3076.2	1677.6	237.2	2599.5	3553.0	934.2	9967.1

[Table/Fig-10]: Mean proteinuria values per number of crosses in the Urocolor strip.

It immediately emphasizes the fact that proteinuria does not increase in relation to crosses and that comparison is not significant [Table / Figure 11].

Groups of comparison per urocolor positive crosses	Mann-Whitney U-test	W of Wilcoxon	Z	Sig.
None vs. 1	203	434	-0.905	0.366
None vs. 2	36	267	1.073	0.283
None vs. 3	7	238	-0.552	0.581
1 vs. 2	56	332	-0.090	0.928
1 vs. 3	10	11	-0.217	0.828
2 vs. 3	2	3	-0.293	0.770

[Table/Fig-11]: Comparison of proteinuria per urocolor positive crosses.
Sig: Significance

DISCUSSION:

As mentioned, the proportion of cesarean rates exceeded the expected international targets, 44 cases (88%). This is a widely discussed national trend. Nowadays, since many hospitals are part of the international criteria for preeclampsia, it includes the use of a urine stick in the comprehensive assessment of women with hypertensive disorders of pregnancy. The urine bar is cheap and easy to use, and the result of the application of this source is that it determines an approximate protein concentration, but is not an absolute value. The patient's hydration condition may affect the result; in fact, in urine samples with a density of <10 10 or in the presence of high salt concentrations, a very negative proteinuria or proteinuria that does not depend on the presence of albumin may produce a false negative result[11]. All these factors mean that the urine probe is weakly correlated with the 24-hour proteinuria assay; this leads to a low diagnostic yield as an important proteinuria indicator. The analysis of many studies investigating the safety of the urine dipstick showed low sensitivity and specificity, resulting in poorly diagnosed or poorly diagnosed preeclampsia[12]. This last sentence is consistent with our results because the urine bar is negative in some cases of severe proteinuria identified by 24-hour urine collection and congo red. Therefore, there was a bad relationship between the severity of the disease and the urocolor strip. The determination of the proteinuria value in a 24-hour urine sample with a dipstick is widely used, although it is insufficient compared to protein deficiency. Furthermore, the absence of proteinuria in the test strip may have excluded the presence of significant proteinuria in patients[13]. Another alternative to the presence of proteinuria was to urinate briefly (8 or 12 hours) for 24 hours sharing the limitations and problems associated with collection of urine. In this paper, 38% of patients had protein3 gm / dL proteinuria, indicating the severity of current cases. In a pilot study based on urinary test, Buhimschi IA et al. Found that 4/35 (11%) of asymptomatic women tested positive for Congo Red before developing preeclampsia[14]. These findings indicate the possibility of developing a test to predict and diagnose preeclampsia requiring only a few drops of urine according to the author. A previous study identified an detection rate of 93% (DR) and 4% false positive rate (FPR). However, RD in urine samples of the first trimester was 33.3%, 16.1% and 20% with 12.8% RPF in early, late and all preeclampsia cases, respectively. Proportion Ratio for preeclampsia was higher than Body Mass Index (BMI) and Mean Blood Pressure (MAP), but lower than previous preeclampsia and black ethnicity. In the first trimester, the Congo Red urine test adds sensitivity to the prediction of preeclampsia in black women undergoing obese, pre-eclampsia, and above average blood pressure. In this pilot study, the positivity with Congo Red was 100%, but we should be aware that the variability observed in positive areas is a problem that can be used for quality and interobserver analysis[15]. Recently, in a study of 101 women over 14 weeks of gestation who underwent an evaluation for pre-eclampsia and diabetes, Parikh L et al. Diabetes gravidity without preeclampsia, even in the presence of first nephropathy. They also found that a CRR of more than 15% correctly classified all

women with preeclampsia and adverse pregnancy outcomes. From this study, it is clear that there is an increase in proteinuria values, two to three positive areas in the 12 to 1 positive area with Congo Red and 1722.2 mg in three to four positive areas. In addition, the statistical comparison between the 2 to 3, 2 to 4 and 3 to 4 positive red areas in the microscope slide showed a significant difference ($p < 0.001$) in relation to the mean proteinuria values in each. In contrast to the insignificance with the ürocolor test, the number of positive crosses in the strip is expected to increase in a heterogeneous manner. Congo Red cost is an important issue. Although it may be excluded from the statistical analysis, it is more expensive than the commercially available urocolor tests, but the second option does not provide data consistent with proteinuria values that put patients at risk of misdiagnosis. A disadvantage of CRR is that this technique cannot differentiate between autoimmune diseases such as lupus nephritis and a hypertensive pregnancy disorder, and this can be applied to other commercially available kits.

CONCLUSION:

This study confirms that women with chronic kidney disease without preeclampsia and preeclampsia have high congophilia urine levels. In this sense, CRR interpreted a positive area that showed a direct relationship to proteinuria levels in the slide. Therefore, the Congo Red test point is a new diagnostic tool for a fast and highly reliable test for the physician working in the obstetric field. This is a test that can be done in any context, even in third world countries, and the results are obtained after 10-15 minutes. In summary, it is expected that the overall evaluation of the misfolded protein load with Congo Red staining would be a simple, effective and highly reproducible diagnostic test. The results are very encouraging to maintain a new research line. In contrast, the urrocolor test is not reliable to decide for kidney damage in pregnancy / puerperium.

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