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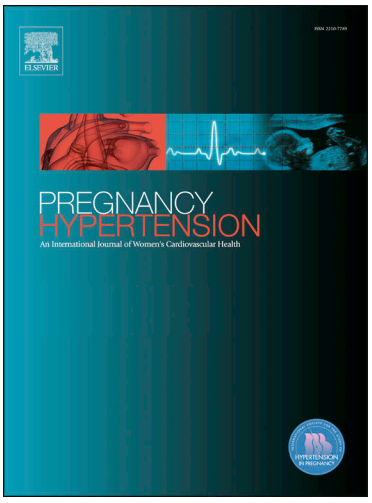

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Congo red dot test in the early prediction and diagnosis of pre-eclampsia in a tertiary health care centre in India

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Abbreviations:
CR – Congo red
CRD - Congo red dot test
CRR - Congo red retention
PE - Pre-eclampsia
sPE – Severe pre-eclampsia
NP-CRL - Normal Pregnant Control
NPV – Negative Predictive Value
PPV – Positive Predictive Value
IUGR – Intra uterine growth Restriction
HELLP - Haemolysis, elevated liver enzymes, low platelet count
FGR – Fetal Growth Restriction
NICU – Neonatal Intensive care

ABSTRACT

Objectives: To demonstrate the use of urine congophilia quantification in the prediction and diagnosis of pre-eclampsia using of Congo red dot test
Study design: A prospective cohort study in 378 consecutive pregnant women was conducted. All eligible, consenting women of gestational age between 10 to 34 weeks were enrolled in the study. The presence of urinary misfolded proteins was screened by a simple dot test technique on unsupported nitrocellulose membrane using Congo red dye.

Results: The urinary congophilia was increased in urine from women with pre eclampsia compared to healthy pregnant controls. The mean CRR value of pre eclamptic pregnant women (35.2 ± 9.4 %) was five times higher than that of mean CRR value of normotensive pregnant women (6.9 ± 4.7 %). The mean gestational age at which Congo red test showed positive was 26.95 ± 2.90 weeks and the time taken from CRD positive to development of PE was 4.92 ± 2.54 weeks of gestation.

Conclusions: In our study, the CRD test was not only effective in predicting pre-eclampsia but was also useful in differentiating between pre-eclampsia and other forms of hypertension, as well as early onset and late onset pre-eclampsia, with positive predictive value of 80.36% and negative predictive value of 92.86%

Keywords: Preeclampsia, Congo red Dot Test, Gestational Hypertension, Congophilia.

1. Introduction

Hypertensive disorders affect around 8-10% of all pregnant women in India [1]. Pre eclampsia (PE) is a life threatening complication of pregnancy characterized by new onset of hypertension and proteinuria after 20 weeks of gestation [2]. It is associated with multi-organ involvement like renal and liver dysfunction, fetal growth retardation, neurological and hematological complications [3]. The incidence of pre-eclampsia is found to be seven times higher in developing countries, as compared to developed countries [4]. Due to difficulty in prediction and timely management, pre-eclampsia continues to contribute largely to maternal and perinatal morbidity and mortality [5]. Recent studies have reported that pre-eclampsia is a protein conformational disorder, similar to Alzheimer's disease, Parkinson's disease and Huntington's disease [6,7]. To this end, it is proposed that amyloid proteins with the propensity to bind to Congo red dye are excreted in the urine of pre-eclamptic women [8]. The pathophysiology of PE is unclear but current research suggests that preeclampsia arises from defective trophoblastic invasion of the maternal uterine spiral arteries leading to placental hypoxia and ischemia [9,10]. The sustained stimulation of the endoplasmic reticulum causes oxidative stress [11] and endothelial dysfunction. The endoplasmic reticulum stress in the placenta leads to up-regulation of the misfolded protein response pathway, which is a common cellular defense mechanism that promotes the removal of these misfolded proteins to prevent

potentially toxic accumulation. This response has been shown in early onset preeclampsia but not in late onset preeclampsia [12,13]. A wide range of potential, novel biomarkers is being investigated for the detection of PE [14]. Congo red is an azo dye, which exhibits a special affinity for misfolded proteins and is used as a gold standard test to identify amyloid fibrils [15]. Recent research has shown that misfolded proteins are present in the urine of pregnant women who develop preeclampsia, well before the onset of clinical symptoms. Studies have also shown that the congo red test is useful for early diagnosis of preeclampsia [16]. The urinary misfolded proteins can be detected by a simple dot test technique on unsupported nitrocellulose membrane using an azo dye like congo red that binds with specific affinity to the β-sheets of amyloid fibrils of misfolded proteins [17,18] which is known as congophilia. The CRD test is being investigated now as an innovative mobile health solution in countries with limited resources, as a diagnostic and prognostic tool for preeclampsia [19]. This study aims to demonstrate the use of urine congophilia quantification in the early prediction and diagnosis of pre-eclampsia by the use of CRD test.

2. Materials and Methods

2.1 Design and study setting

We conducted a prospective cohort study in 378 consecutive pregnant women at Department of Obstetrics and Gynecology, Rajarajeswari Medical College and Hospital, Bangalore. The study was conducted for two years between December 2017 and December 2019. All eligible, consenting women of gestational age between 10 to 34 weeks with singleton pregnancies were approached for enrolment in the study. The exclusion criteria included non-consenting women, history of cardiovascular disease, renal disease, pulmonary disease, neurological disease, history of mental illness, and drug/hormone consumption.

2.2 Assessment of urine congophilia

2.2.1 Sample Collection

Fresh mid-catch urine samples of 5 to 10mL were collected from all eligible patients and processed within 3 hours as reported by Buhimschi et al 2014 [8]. The urine samples were centrifuged at 15000 Xg for 15 minutes at 4 ^{o}C and supernatant was stored at -80 ^{o}C in multiple aliquots for further assays. The protein concentration was

normalized to 6.6 mg/dL and the total protein concentration was quantified using Pierce Bicinchoninic Acid (BCA) Assay Kit (Thermo Scientific, 23227, USA). Each sample was tested in duplicate.

2.2.2 Congo Red Dot (CRD) Test

100µL of normalized urine was mixed with 2 µL of stock (5mg/mL) Congo red aqueous Solution (Sigma, Cat# C6277, USA). 100 µL of phosphate buffered saline (PBS) was mixed with 2 µL of Congo red solution, which served as Blank (BLK). The samples are mixed with vortexer (PTR-35, Grant Bio, UK) for 1 hour at room temperature. Following, 5 µL of mixture was spotted in duplicate onto an unsupported nitrocellulose membrane (0.22 µm, GE Healthcare, USA) and left to air dry for 15 minutes. The membrane was washed with distilled water for 3 minutes at room temperature and then photographed using a digital camera to acquire the first picture. Then, the membrane was washed with increasing concentrations of methanol – (a) 50% methanol for 3 min (b) 70% methanol for 1 min and (c) 90% methanol for 10 min until the red color completely disappears from Blank. During this time, the red color of samples from normal pregnant women washed away or disappeared. In urine samples from women with severe PE, the Congo red dye was retained in the nitrocellulose membrane and the spots remained visibly red, due to the presence of misfolded proteins. The photograph was taken using digital camera to acquire a second picture for assessment of CRD test. The analysis was performed with Image J software. The background of each image obtained from blank control was subtracted and the density of inverted image on the black and white axis was measured to obtain Congo red retention. CRR was calculated by taking a ratio of intensity of the spot after wash and before wash and expressed as a percentage.

2.3 Statistical Analysis

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented as Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (Inter group analysis) on metric parameters. Leven's test for homogeneity of

variance has been performed to assess the homogeneity of variance. Chi-square or Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups, Non-parametric setting for Qualitative data analysis. The Statistical software namely SPSS 22.0, and R environment ver.3.2.2 were used for the analysis of the data and Microsoft word and Excel were used to generate graphs and tables. P value of ≤0.05 was considered statistically significant.

3. Results

We recruited 378 pregnant women for the prospective cohort study for an early prediction of Pre eclampsia using congo red dot test (CRD). The data derived from CRD test (Fig. 1) was analyzed and the results were compared with clinical data to determine the significance of CRD test in prediction of PE. The demographic characteristics of study population and various parameters like interval between CRD positive and onset of disease, maternal complications and fetal complications (fetal growth restriction (FGR) and Intrauterine fetal demise), are analysed and found to be statistically significant (p=0.001). The foetal and maternal complication rate between the CRD positive and CRD negative group was statistically significant (p value <0.001) **(Table 1).** We found significantly increased CRD positive test in subjects with higher systolic and diastolic blood pressure (P<0.001). We examined prediction of PE using CRD test at early onset versus late onset. At early onset of PE (<34 weeks), the CRD test positive was 87.5%, whereas at late onset of PE only 24% of cases showed positive for CRD test (P<0.001) **(Table 1)**. Based on Chi square test, there was significantly higher association between PE and positivity of CRD test (P<0.001), which shows that the CRD test could be a good predictor of PE at early onset of the disease **(Table 2)**. The sensitivity and specificity of the CRD test to predict PE was found to be 66.2% and 96.4% respectively. The positive prediction value (PPV) and negative predictive value (NPV) for CRD test was found to be 80.36% and 92.8% **(Table 2)**. The mean gestational age at which Congo red test became positive was 26.95 ± 2.90 weeks and the time taken from CRD positive to development of PE was 4.92 ± 2.54 weeks of gestation **(Table 3)**.

The urinary protein concentration was significantly increased in pre-eclamptic women compared to normal patients. The urinary protein concentration in PE samples was 123.8 ± 14.4 mg/ml as compared to 5.64 ± 0.60 in non-PE samples (Fig.2A). The

affinity of CR bound protein was greatly increased in pre-eclamptic women compared to non pre eclamptic women and the mean value of CR bound protein in the spectrophotometric assay of PE and non-PE samples was 0.0569 ± 0.007 and 0.0029 ± 0.003 respectively (Fig.2B). The urinary congophilia was increased in urine from women with pre eclampsia compared to healthy pregnant controls. The mean congo red retention (CRR) value of urinary congophilic protein from pre eclamptic women (35.2 ± 9.4%) was five times higher than that of normotensive pregnant women (6.9 ± 4.7%), which is statistically significant ($p < 0.001$; Fig.2C).

Discussion

The limited knowledge on the etiology, pathogenesis and the gravity of pre-eclampsia, makes it a rare orphan disease that is difficult to tackle, often with, disastrous consequences [20]. Considering that the disease process starts much earlier than the clinical manifestations, there is a pressing need for a functional, simple, effective and inexpensive screening system that will detect the disease in its nascent stage and make the diagnosis and management more cohesive, especially in low resource settings. Recent studies have indicated that pre-eclampsia is a protein conformational disorder with protein instability and misfolding [8]. This is similar to the pathogenesis of diseases like Huntington's, Parkinson's and Alzheimer's disease [6]. To this end, it is proposed that misfolded proteins may be excreted in the urine of pre-eclamptic women much earlier than the clinical manifestations of the disease, and these proteins have the propensity to bind to special dyes like the Congo red [21]. Hence, Congo red testing has the potential to be the blanket screening test that will expedite the time of diagnosis of the disease, thereby enabling us to provide timely management. Soluble, immature amyloid oligomers from different amyloid forming sequences are recognised as the primary toxic species responsible for the pathogenesis of pre-eclampsia [8]. The urinary misfolded amyloid proteins can be detected by a simple dot test technique on unsupported nitrocellulose membrane using an azo dye, like Congo red, that has special affinity for beta sheets of the amyloid fibrils of misfolded proteins. This is called urinary congophilia [17,18].

The clinical relevance of our research is that, urine congophilia can serve as a rapid diagnostic and prognostic marker for PE, where other tests like PlGF, sFlt-1/PlGF ratio, placental protein 13, soluble endoglin and PAPP-A require sophisticated

equipment and are also time consuming and costly [22]. It is well known that proteinuria and blood pressures are not ideal tests to discriminate pre-eclampsia from other variants of hypertension, which have different etiologies [23]. The poor performance of dipsticks and 24 hour urine protein estimation has been proven beyond doubt in a study by Thangarathinam et al [24]. The dipstick colorimetric reagent (tetrabromophenol blue) is said to detect only a small subset of proteins excreted in urine of pre-eclamptic women. Two components (*k*FLCs and fragmented albumin) that have been identified in the congophilic precipitate from urine samples of PE patients failed to produce any dipstick reaction [25]. Hence the CRD test proved to be far superior in this regard. In a previous study by Nagarajappa et al, results showed that there is a significant difference in the level of urinary congophilia between early onset and late onset PE and congophilia is not affected by factors such as gestational age of onset, severity or superimposed eclampsia [26]. There is evidence that early onset and late onset PE are distinct entities with placental factors being the cause in early onset while late onset PE is attributed to maternal factors [27]. Recent studies have shown that activation of Unfolded Response Pathway in the placenta is different between early onset and late onset PE [13,28]. The maternal factor that leads to urinary misfolded protein appears to be shared by both early-onset and late-onset pre-eclampsia and thus represents a core pathophysiological event [26,28].

In our study, the CRD test was not only effective in predicting pre-eclampsia but was also useful in differentiating between pre-eclampsia and other forms of hypertension, as well as early onset and late onset pre-eclampsia. The sensitivity and the specificity of the test was 66.18% and 96.45% respectively. The positive predictive value (PPV) and negative predictive value (NPV) was 80.36% and was 92.86% respectively, thus making this test a valuable tool for the prediction of pre-eclampsia. Our study has confirmed the presence of congophilia in PE women by 4-7 weeks prior to the onset of clinical symptoms. The translational relevance of our research is that urine congophilia can be used as rapid test in prediction of PE and also as a diagnostic marker prior to the clinical manifestation of PE. In conclusion, our study demonstrates that the Congo red dot test could provide a solution to bridge the gaps in current tools available for the early diagnosis and prediction of pre-eclampsia in low-

income settings, by being an inexpensive, accurate, minimally invasive, rapid and easy to perform test, with a good specificity and sensitivity.

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References

[1] M. Sajith, V. Nimbargi, A. Modi, R. Sumariya, A. Pawar, Incidence of pregnancy induced hypertension and prescription pattern of antihypertensive drugs in pregnancy, Int J Pharma Sci Res. 23 (2014) 4.

[2] ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. American College of Obstetricians and Gynecologists, Int. J. Gynaecol. Obstet. Off. Organ Int. Fed. Gynaecol. Obstet. 77 (2002). https://pubmed.ncbi.nlm.nih.gov/12094777/ (accessed April 12, 2021).

[3] M. Ben W J, R. Claire T, T. Shakila, M. Laura A, de G. Christianne J M, Justus G Hofmeyr, Pre-eclampsia, The Lancet. 387 (2016) 999–1011. https://doi.org/10.1016/S0140-6736(15)00070-7.

[4] A. Malik, B. Jee, S.K. Gupta, Preeclampsia: Disease biology and burden, its management strategies with reference to India, Pregnancy Hypertens. 15 (2019) 23–31. https://doi.org/10.1016/j.preghy.2018.10.011.

[5] A. Jeyabalan, Epidemiology of preeclampsia: impact of obesity, Nutr. Rev. 71 (2013) S18–S25. https://doi.org/10.1111/nure.12055.

[6] P. Sweeney, H. Park, M. Baumann, J. Dunlop, J. Frydman, R. Kopito, A. McCampbell, G. Leblanc, A. Venkateswaran, A. Nurmi, R. Hodgson, Protein misfolding in neurodegenerative diseases: implications and strategies, Transl. Neurodegener. 6 (2017) 1–13. https://doi.org/10.1186/s40035-017-0077-5.

[7] H. Inouye, D.A. Kirschner, Alzheimer's β-Amyloid: Insights into Fibril Formation and Structure from Congo Red Binding, in: Alzheimer's Dis., Springer, Boston, MA, 2005: pp. 203–224. https://doi.org/10.1007/0-387-23226-5_10.
[8] Buhimschi Ia, Nayeri Ua, Zhao G, Shook Ll, Pensalfini A, Funai Ef, Bernstein Im, Glabe Cg, Buhimschi Cs, Protein misfolding, congophilia, oligomerization, and defective amyloid processing in preeclampsia, Sci. Transl. Med. 6 (2014). https://doi.org/10.1126/scitranslmed.3008808.
[9] N. Al-Jameil, F. Aziz Khan, M. Fareed Khan, H. Tabassum, A Brief Overview of Preeclampsia, J. Clin. Med. Res. 6 (2014) 1–7. https://doi.org/10.4021/jocmr1682w.
[10] Mustafa R, Ahmed S, Gupta A, Venuto Rc, A comprehensive review of hypertension in pregnancy, J. Pregnancy. 2012 (2012). https://doi.org/10.1155/2012/105918.
[11] J.W. Meekins, R. Pijnenborg, M. Hanssens, I.R. MCFadyen, A. van Asshe, A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies, BJOG Int. J. Obstet. Gynaecol. 101 (1994) 669–674. https://doi.org/10.1111/j.1471-0528.1994.tb13182.x.
[12] Wu C, Wang Z, Lei H, Zhang W, Duan Y, Dual binding modes of Congo red to amyloid protofibril surface observed in molecular dynamics simulations, J. Am. Chem. Soc. 129 (2007). https://doi.org/10.1021/ja0662772.
[13] Lenna S, Han R, Trojanowska M, Endoplasmic reticulum stress and endothelial dysfunction, IUBMB Life. 66 (2014). https://doi.org/10.1002/iub.1292.
[14] S. Grill, C. Rusterholz, R. Zanetti-Dällenbach, S. Tercanli, W. Holzgreve, S. Hahn, O. Lapaire, Potential markers of preeclampsia – a review, Reprod. Biol. Endocrinol. 7 (2009) 70. https://doi.org/10.1186/1477-7827-7-70.
[15] Buell Ak, Dobson Cm, Knowles Tp, Welland Me, Interactions between amyloidophilic dyes and their relevance to studies of amyloid inhibitors, Biophys. J. 99 (2010). https://doi.org/10.1016/j.bpj.2010.08.074.
[16] W.E. Klunk, J.W. Pettegrew, D.J. Abraham, Quantitative evaluation of congo red binding to amyloid-like proteins with a beta-pleated sheet conformation.:, J. Histochem. Cytochem. 37 (1989) 1273–81. https://doi.org/10.1177/37.8.2666510.
[17] McCarthy Fp, Adetoba A, Gill C, Bramham K, Bertolaccini M, B. Gj, G. G, S. Pt, P. L, C. Lc, Urinary congophilia in women with hypertensive disorders of pregnancy and preexisting proteinuria or hypertension, Am. J. Obstet. Gynecol. 215 (2016). https://doi.org/10.1016/j.ajog.2016.04.041.
[18] B. Stopa, B. Piekarska, L. Konieczny, J. Rybarska, P. Spólnik, G. Zemanek, I. Roterman, M. Król, The structure and protein binding of amyloid-specific dye reagents., Acta Biochim. Pol. 50 (2003) 1213–1227. https://doi.org/10.18388/abp.2003_3645.
[19] S.M. Jonas, T.M. Deserno, C.S. Buhimschi, J. Makin, M.A. Choma, I.A. Buhimschi, Smartphone-based diagnostic for preeclampsia: an mHealth solution for administering the Congo Red Dot (CRD) test in settings with limited resources, J. Am. Med. Inform. Assoc. 23 (2016) 166–173. https://doi.org/10.1093/jamia/ocv015.
[20] S. Hahn, Preeclampsia – Will Orphan Drug Status Facilitate Innovative Biological Therapies?, Front. Surg. 2 (2015). https://doi.org/10.3389/fsurg.2015.00007.

[21] R.P. Linke, Highly sensitive diagnosis of amyloid and various amyloid syndromes using Congo red fluorescence, Virchows Arch. 436 (2000) 439–448. https://doi.org/10.1007/s004280050471.
[22] V. Pant, B.K. Yadav, J. Sharma, A cross sectional study to assess the sFlt-1:PlGF ratio in pregnant women with and without preeclampsia, BMC Pregnancy Childbirth. 19 (2019) 1–8. https://doi.org/10.1186/s12884-019-2399-z.
[23] F.H. Harlow, M.A. Brown, The Diversity of Diagnoses of Preeclampsia, Hypertens. Pregnancy. 20 (2001) 57–67. https://doi.org/10.3109/10641950109152642.
[24] S. Thangaratinam, A. Coomarasamy, F. O'Mahony, S. Sharp, J. Zamora, K.S. Khan, K.M. Ismail, Estimation of proteinuria as a predictor of complications of pre-eclampsia: a systematic review, BMC Med. 7 (2009) 10. https://doi.org/10.1186/1741-7015-7-10.
[25] Maisnar V, Tichy M, Stulik J, Vavrova J, Friedecky B, Palicka V, Spirkova J, Zaloudkova L, Hernychova L, Spacilova J, Buchler T, Hajek R, The problems of proteinuria measurement in urine with presence of Bence Jones protein, Clin. Biochem. 44 (2011). https://doi.org/10.1016/j.clinbiochem.2011.01.008.
[26] Nagarajappa. C, Rangappa. S.S, Suryanarayana. R, Balakrishna. S, Urinary congophilia in preeclampsia: Experience from a rural tertiary-care hospital in India, Pregnancy Hypertens. 13 (2018). https://doi.org/10.1016/j.preghy.2018.05.006.
[27] Stergiotou I, Crispi F, Valenzuela-Alcaraz B, Bijnens B, Gratacos E, Patterns of maternal vascular remodeling and responsiveness in early- versus late-onset preeclampsia, Am. J. Obstet. Gynecol. 209 (2013). https://doi.org/10.1016/j.ajog.2013.07.030.
[28] Yung Hw, Atkinson D, Campion-Smith T, Olovsson M, Charnock-Jones Ds, Burton Gj, Differential activation of placental unfolded protein response pathways implies heterogeneity in causation of early- and late-onset pre-eclampsia, J. Pathol. 234 (2014). https://doi.org/10.1002/path.4394.

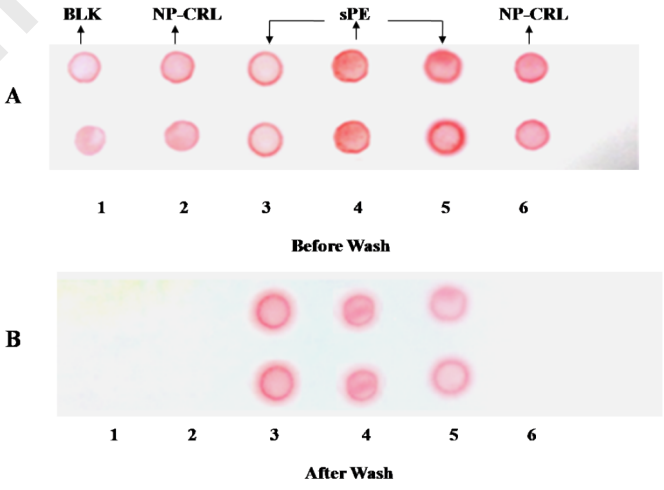

Figure 1. Representative Figure of CRD test: A. Urine from sPE women (lanes 3 to 5) and Normal Pregnant Control (NP-CRL) (lanes 2 and 6), Blank (lane 1), B. Nitrocellulose membrane blot showing positive result of CRD test in sPE samples after washing with increased concentration of methanol.

Table 1: Association of clinical parameters and Congo red test of study population

Parameters	Congored test results		Total (n=378)	P value *
	Negative (n=322)	Positive (n=56)		
Age in years				
18-24	161(50%)	19(33.9%)	180(47.6%)	0.158
25-29	123(38.2%)	29(51.8%)	152(40.2%)	
30-34	31(9.6%)	7(12.5%)	38(10.1%)	
35-40	7(2.2%)	1(1.8%)	8(2.1%)	
SBP (mm Hg)				
<120	176(54.7%)	6(10.7%)	182(48.1%)	<0.001
120-140	128(39.8%)	18(32.1%)	146(38.6%)	
>140	18(5.6%)	32(57.1%)	50(13.2%)	
DBP (mm Hg)				
<80	167(51.9%)	5(8.9%)	172(45.5%)	<0.001
80-100	150(46.6%)	38(67.9%)	188(49.7%)	
>100	5(1.6%)	13(23.2%)	18(4.8%)	
Parity				
Primi	174(54%)	33(58.9%)	207(54.8%)	0.497
Multi	148(46%)	23(41.1%)	171(45.2%)	
Onset of PE				
Early (<34 weeks)	5 (12.5%)	35 (87.5%)	40 (10.6%)	<0.001
Late (>34 weeks)	17 (73%)	11 (24%)	28 (7.4%)	
Complication				
IUD	2(0.6%)	2(3.6%)	4(1%)	0.046
IUGR	29(9%)	26(46.4%)	55(14.5%)	<0.001
NICU ADMISSIONS	20(6.2%)	23(41.1%)	43(11.3%)	<0.001
NEONATAL DEATH	3(0.9%)	1(1.8%)	4(1%)	0.564
ECLAMPSIA	0(0%)	1(1.8%)	1(0.27%)	0.016
HELLP	0(0%)	1(1.8%)	1(0.27%)	0.016

* Fisher Exact Test

Table 2: Accuracy of the CRD test as a predictor for PE

Developed PE	Congored test results		Total
	Negative	Positive	
Nil	299(92.9%)	11(19.6%)	310(82%)
Yes	23(7.1%)	45(80.4%)	68(18%)

Total	322(100%)	56(100%)	378(100%)

Chi-Square Test Accuracy=91.01% (P<0.001)

Sensitivity	Specificity	PPV	NPV
66.18	96.45	80.36	92.86

Table 3: Relationship between CRD test, Gestational Age and PE Development

Particulars	Period of gestation (weeks)
Gestation age at which CRD became positive	26.35 ± 2.90
Gestational age at development of PE	31.20 ± 3.38
Period from CRD positive testing to PE development	4.92 ± 2.54

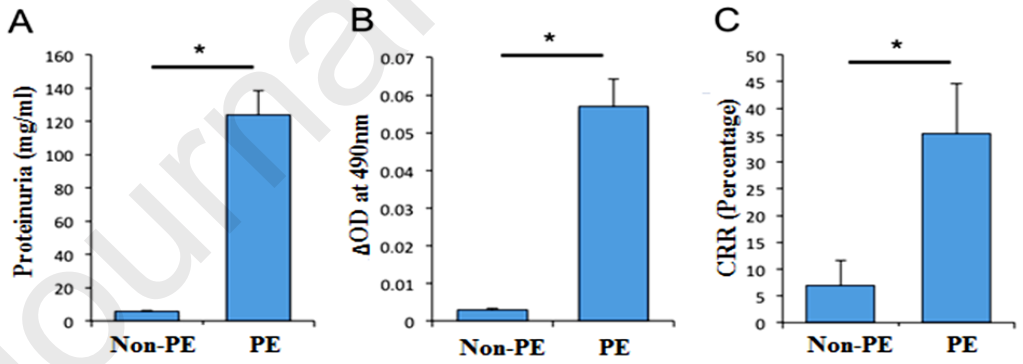

Figure.2. A. Urinary protein concentration (mg/ml) in preeclampsia (PE) and normal pregnancy (Non-PE) by urinalysis dipstick and quantified using BCA Assay method. B. Comparison of CR-bound protein quantity after normalization using spectrophotometric reading at 490nm (OD). C. Congo red retention rate (percentage) of PE samples versus normal control samples. * p<0.001

Highlights of the manuscript titled “Congo red dot test in the early prediction and diagnosis of pre-eclampsia in a tertiary health care centre in India”

1. This is one of largest single Institute cohort study (N=378) in India extensively to determine the feasibility and efficiency of the Congo red dot (CRD) test under Indian tertiary clinical setup.

2. We used CRD test for early prediction of preeclampsia in Indian pregnant women and compared the results with the clinical data to determine the correlation.