



# A Study on the Role of Urinary Congophilia in Early Detection of Preeclampsia

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## Abstract

**Introduction** Preeclampsia and eclampsia are important causes of maternal morbidity. Preeclamptic women secrete misfolded proteins in the urine. Buhimschi et al had developed a new test for diagnosis of preeclampsia. This test is based on staining of misfolded protein with Congo red dye. Misfolded proteins are derived from syncytiotrophoblast microparticles (STBMs). These STBM are membrane bound vesicles and contain misfolded proteins. In preeclampsia, glomeruli of kidneys are disrupted and these damaged protein reach the urine.

**Aim and Objective** This study aimed to investigate the role of urinary congophilia in early prediction of preeclampsia.

**Materials and Methods** This test was done in 250 pregnant women attending the Gynaecological Outpatient Department. Urine sample of early morning was taken and test was done in the Department of Biochemistry. The included pregnant women were of gestational age between 14 and 18 weeks. The staining of urine with Congo red dye was done and washed with methanol. The retention of dye was interpreted with naked eye. The more retention of dye, the more chances of developing preeclampsia later. The patients were followed-up till delivery. The patients who developed preeclampsia later part of pregnancy were recorded. Mean arterial pressure (MAP) and past history and body mass index were also recorded.

**Results** Out of 250 patients, 30 developed preeclampsia later. A total of 34 patients were having positive urinary congophilia and only 20 patients developed preeclampsia later. MAP more than 90 mm Hg is abnormal but 66.7% of patients who developed preeclampsia had MAP >90 mm Hg. In 16.7% of patients, who developed preeclampsia later, had positive past history of hypertension. In 66.7% of patients, who were positive for urinary congophilia, later developed preeclampsia.

**Conclusion** Preeclampsia and eclampsia are important causes of maternal mortality and morbidity. So, early detection can prevent complications and timely management. Urinary congophilia is one of such test which can help in early prediction of preeclampsia. If it is combined with past maternal history and MAP, it gives more good results. The detection rate is much higher if signs and symptoms of preeclampsia are noticed timely.

## Keywords

- ▶ preeclampsia
- ▶ urinary congophilia
- ▶ misfolded protein
- ▶ biomarkers
- ▶ Congo red

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## Introduction

Preeclampsia is one of the leading causes of maternal morbidity and mortality.<sup>1</sup> The definition of preeclampsia according to The American College of Obstetricians and Gynecologists (ACOG) is the presence of hypertension and proteinuria after 20 weeks of gestation. But in revised definition by the ACOG (2013) task force states that proteinuria may or may not present.<sup>2</sup> Preeclampsia can affect 5 to 7% of all pregnant ladies, cause 70,000 maternal deaths globally, and also 500,000 fetal deaths.<sup>3-5</sup> Preeclampsia if left untreated, it can progress to eclampsia which is associated with complications like renal failure, seizures, pulmonary edema, disseminated intravascular coagulation (DIC), and can lead to death as well. The definitive treatment for severe preeclampsia and eclampsia is delivery of placenta and baby. The early prediction of preeclampsia is very important, so that timely treatment can be started and complications can be avoided or decreased.

For the last two decades, there are lots of studies to understand the pathophysiology of preeclampsia, and misfolded protein is a new concept for preeclampsia.<sup>6</sup> There are various biochemical and biophysical markers which can predict preeclampsia before the clinical symptoms appear. These markers are pregnancy-associated plasma protein A (PAPP-A), placental growth factor, and placental protein 13 and others.<sup>7-9</sup> An effective combine method for prediction of preeclampsia in first trimester is based on maternal history, mean arterial pressure (MAP), uterine artery pulsatility index, and biochemical markers. But still there is a need of simple, cost effective, and noninvasive method for early prediction.

It is seen in many new studies that misfolded proteins are found in various body fluids of preeclamptic women. These proteins can be found in the urine of preeclamptic women as early as 10 weeks of gestation<sup>10</sup> as reported by Buhimschi et al.

Buhimschi et al had developed a test based on these misfolded proteins which are secreted in urine of preeclamptic women.<sup>11-13</sup> Misfolded proteins bind to Congo red dye. This is known as urinary congophilia which is also used to diagnose Creutzfeldt-Jakob disease<sup>14</sup> as described by Halimi et al.

The mechanism of binding of Congo red to amyloids is not clear. A study by Frid et al suggested that Congo red has affinity to bind proteins which are rich in  $\beta$  sheets.<sup>15</sup> Rood et al<sup>16</sup> also suggested that Congo red dot paper test is a simple, noninvasive test for diagnosis and prediction of preeclampsia.

## Materials and Methods

This study was conducted at the Department of Obstetrics and Gynaecology in collaboration with the Department of Biochemistry of Banaras Hindu University. Ethical clearance was taken from the Institute Ethical Committee.

## Inclusion Criteria

Patients were included with gestational age between 14 and 18 weeks. Sample size was 250.

## Procedure

Early morning urine sample was collected from pregnant women attending the Gynaecological Outpatient Department.

The sample was transported to the biochemistry laboratory. Congo red was freshly prepared by taking 5 mg of dry Congo red dye and mixing it with 10 ml of distilled water. So, it became 0.5 mg/mL. It was thoroughly mixed by shaker.

Nitrocellulose membrane was taken and of size 6 cm  $\times$  4 cm. Urine sample was taken with pipette and 10  $\mu$ L was put on nitrocellulose membrane. It was dried and then staining was done with Congo red dye and incubated for 1 hour. Then to remove extra dye, it was washed with methanol (50, 70, and 90%).

The value of Congo red retention after wash with methanol is used as a diagnostic indicator. Interpretation was done by visual examination of nitrocellulose paper. Then depending on concentration, they are divided into congophilia positive or negative. Data were recorded in an excel sheet. Statistical analysis was done using the SPSS software, version 20.

Follow-up of these patient was done till delivery. Patients who had past positive history of preeclampsia were not given low-dose aspirin. Those patients who developed preeclampsia later in pregnancy were noted. Then data were correlated with the first time urinary congophilia-tested patients.

We recorded the number of patients with congophilia positive at the time of recruitment who developed preeclampsia and also the percentage of congophilia-positive patients who did not develop preeclampsia.

## Results

The observations made from this study are listed in this section. A total of 250 pregnant cases of urinary congophilia were tested and among these, 30 cases developed preeclampsia later, during follow-up.

Development of Preeclampsia was seen maximum in age group 20 to 24 (36.7%) and 25 to 29 (33.3%) as presented in **Table 1**.

MAP >90 mm Hg is abnormal. As described in **Table 2**, 66.7% of patients developed preeclampsia who had MAP

**Table 1** Age

Age group (y)	Development of preeclampsia		Total
	No	Yes	
15-19	5 (2.3%)	0 (0%)	5
20-24	73 (33.2%)	11 (36.7%)	84
25-29	88 (40%)	10 (33.3%)	98
30-35	40 (18.2%)	09 (30%)	49
>35	14 (6.4%)	0 (0%)	14
Total	220	30	250

Note:  $n = 250$ ,  $\chi^2 = 4.867$ , and  $p = 0.301$ .

**Table 2** Mean arterial pressure

Mean arterial pressure	Development of preeclampsia		Total
	No	Yes	
<90 mm Hg	99 (45%)	10 (33.3%)	109 (43.6%)
>90 mm Hg	121 (55%)	20 (66.7%)	141 (56.4%)
Total	220	30	250

Note:  $\chi^2 = 1.461$ ,  $p = 0.227$ .

>90 mm Hg and 33.3% had MAP <90 mm Hg but still developed preeclampsia.

In **Table 3**, it is seen that 53.3% of patients who developed preeclampsia had body mass index (BMI) between 18.5 and 23 kg/m<sup>2</sup> and also, 33.3% of patients who developed preeclampsia had BMI >25 kg/m<sup>2</sup>.

**Table 3** Body mass index

Body mass index (kg/m <sup>2</sup> )	Development of preeclampsia		Total
	No	Yes	
13–18.5	11 (5%)	1 (3.3%)	12 (4.8%)
18.5–23	86 (39.1%)	16 (53.3%)	102 (40.8%)
23–25	58 (26.45%)	03 (10%)	61 (24.4%)
>25	65 (29.55%)	10 (33.3%)	75 (30%)
Total	220	30	250

Note:  $\chi^2 = 4.489$ ,  $p = 0.213$ .

**Table 4** Past history of preeclampsia/eclampsia

Past h/o preeclampsia/eclampsia	Development of preeclampsia		Total	Values with 95% confidence interval
	No	Yes		
Absent	220 (100%)	25 (83.3%)	245 (98%)	PPV = 100 (57.8–100) NPV = 89.8% (46.7–89.8) Sensitivity = 16.7 (9.6–16.7) Specificity = 100 (99–100) Accuracy = 90 (88.3–90)
Present	0	05 (16.7%)	05 (2%)	
Total	220	30	250	

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

**Table 5** Congophilia positive and development of preeclampsia

Urinary congophilia	Development of preeclampsia		Total	Values with 95% CI
	No	Yes		
Negative	206 (93.6%)	10 (33.35%)	216 (86.4%)	PPV = 58.8 (45.7–69.4) NPV = 95.4% (93.3–97) Sensitivity = 66.7% (51.8–78.6) Specificity = 93.6% (91.6–95.3) Accuracy = 90.4% (86.8–93.3)
Positive	14 (6.45%)	20 (66.7%)	34 (13.6%)	
Total	220	30	250	

Abbreviations: CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

In **Table 4**, it shows that 16.7% of patients who develop preeclampsia had positive past history of hypertension. Rest 83.3% of patients who developed preeclampsia did not have past history of hypertension. The positive predictive value was 100 and negative predictive value was 89.8.

In **Table 5**, it is seen that 66.7% of patients who developed preeclampsia were congophilia positive and 33.5% of patients who were congophilia negative also developed preeclampsia. The positive predictive value was 58.8 and negative predictive value was 95.4.

**Table 6** shows binary logistic regression which described that congophilia-positive patients had 28 times more risk of developing preeclampsia. The patient with increased MAP has 1.27 times increase risk of developing preeclampsia.

## Discussion

Preeclampsia and eclampsia are two of the three important causes of maternal mortality. In developed countries, maternal mortality due to preeclampsia has decreased as compared with developing countries. In developing countries, it is still high due to various factors like lack of antenatal care, poverty, lack of timely access to critical care.<sup>17</sup> It has been seen that 10 to 15% of direct maternal deaths are due to preeclampsia and eclampsia as described by Duley.<sup>18</sup> Preeclampsia can lead to complications like liver failure, pulmonary edema, renal failure, and DIC.

There are some points to note from this study listed below:

- In our study, 30 out of 250 patients develop preeclampsia, so the chance of developing preeclampsia was 12%.
- A previous pregnancy with preeclampsia is a risk factor in parous women. For example, in a study by Poon et al, the incidence of preeclampsia was 42% in those with such a history

**Table 6** Binary logistic regression analysis of development of preeclampsia on congophilia and mean arterial pressure

Factors	Development of pre-eclampsia Yes (30) no (220)	Sig.	Odds ratio	95% confidence interval
Congophilia				
Positive	20 (66.7) 14 (6.4)	0.000	28.731	11.273 73.225
Negative	10 (33.3) 206 (93.6)			
Mean arterial pressure (mm Hg)				
> 90	121 (55.0) 20 (66.7)	0.622	1.275	0.485–3.355
≤90	99 (45.0) 10 (33.3)			

Note:  $R^2 = 0.390$ ,  $p = 0.000$ .

compared with only 4.9% without previous history of preeclampsia.<sup>19</sup> In our study, the incidence was 16.7% who had positive past history of preeclampsia. It has been shown by Bartsch et al<sup>20</sup> that the past history of preeclampsia increases the risk of preeclampsia in future pregnancies by eight times.

- It has been seen in the study done by Rodriguez-Chávez et al that Congo red dye has high affinity for beta-folded proteins of preeclampsia.<sup>21</sup> It has also been shown that Congo red staining of misfolded proteins in preeclampsia is simple, efficient, and reproducible test, and a new line of research can be done.
- Nagarajappa et al had shown in their study that the urinary congophilia is present in Indian women with preeclampsia and it is a good screening test.<sup>22</sup> Our study also confirms that congophilia is a good screening test and it gives better results if other parameters, like maternal history and MAP, are taken into consideration.
- According to Sammar et al, the urinary congophilia adds to the accuracy of preeclampsia prediction in first trimester.<sup>23</sup> The odds ratio for Congo red alone was superior than BMI and MAP.

### Limitation of Study

- This kit is not available in India and also not available through online purchase.

### Conclusion

Preeclampsia and eclampsia are major cause of maternal mortality. Urinary congophilia is a new screening test for preeclampsia. It has started a new research of role of misfolded proteins in preeclampsia. If it is combined with maternal history and MAP, the early detection is increased and complications can be decreased.

#### Funding

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#### Conflict of Interest

None declared.

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