ABSTRACT

Ultraviolet Spectroscopy vs Congo Red Card Test—A Step Forward toward Early Prediction of Preeclampsia: A Case–Control Study

Neena Gupta[1], Garima Gupta[2], Uruj Jahan[3], Sonali Bisht[4]

Abstract

Aim: Comparative evaluation of ultraviolet–visible (UV–Vis) spectroscopy and Congo red card-based test in the diagnosis of preeclampsia.

Materials and methods: In this case–control study, UV–Vis spectroscopy and Congo red card test were done using mid-stream clean catch urine samples collected from 98 cases and 98 controls. The results obtained were used to calculate the specificity and sensitivity of each test.

Results: Out of 98 cases, 76 showed increased absorbance in UV–Vis spectroscopy while 69 showed Congo red positivity. Thus, the ultraviolet (UV)-absorbance method was found to be more sensitive (77.53) and specific (80.61) compared to the Congo red paper-based test (sensitivity, 70.40; specificity, 64.28) in the detection of the misfolded proteins present in the urine samples of the preeclamptic patients.

Conclusion: The UV–Vis absorption spectroscopy is a renowned yet innovative technique in the diagnosis of preeclampsia. It is more sensitive and specific to the existing Congo red paper-based test.

Clinical significance: Several studies show that urine of preeclampsia patients shows the presence of misfolded proteins which are present about 2 weeks before the onset of symptoms, that is, clinical diagnosis. This creates scope for early detection and diagnosis of preeclampsia. In this study, we establish the role of spectroscopy as a novel test in diagnosing misfolded proteins in preeclamptic patients and compare its diagnostic performance to the highly acclaimed Congo red card test.

Keywords: Adverse maternal outcome, Congophilia, Congo red paper test, Misfolded proteins, Preeclampsia, Prevention, Spectroscopy.

Journal of South Asian Federation of Obstetrics and Gynaecology (2024): 10.5005/jp-journals-10006-2325

Introduction

Over 60,000 maternal mortalities are attributed to pregnancy hypertension and related disorders in the world per year.[1] In India, the incidence of preeclampsia (PE) is reported to be 8–10%.[2]

Major contributors to etiopathogenesis of PE are hypoxic injury and release of inflammatory cytokines which can ultimately lead to protein misfolding.[3–5] Therefore, these conditions lead to aggregation and deposition of misfolded proteins in the placenta and body fluids such as urine. The "foldedness" and stability of the structure of proteins depend upon their three-dimensional structure. In this arrangement, the hydrophobic residues are buried in the protein core. On exposure to stress, hypoxia, and other conditions responsible for the pathogenesis of PE, the proteins lose their structural stability leading to misfolding and thereby exposure of these hydrophobic ends to the outer microenvironment. These residues mostly include aromatic amines such as tryptophan, tyrosine, and phenylalanine which have the ability to absorb light in the near-ultraviolet (UV) region (240–295 nm) when measured by ultraviolet–visible (UV–Vis) spectroscopy.[6] These changes in the protein structure can be indirectly measured by UV–Vis spectroscopy in the form of increased absorbance in this region. This property is used to detect misfolded proteins in the urine of PE patients. Spectroscopy is a unique and novel method in the detection of preeclampsia and can be a game changer due to its cost effectiveness, convenience, and speed.

Several researches show the accumulation of amyloid precursor protein fragments and Ab amyloid plaques in the placenta, along with the presence of proteins such as α-1-antitrypsin, albumin, immunoglobulin G kappa (IgGκ)-free light chain, ceruloplasmin, and interferon-inducible protein 6-16 in different bodily fluids including urine of PE patients.[7] The Congo red dye (CRD) is widely used to demonstrate the deposition of amyloid plaques in diseases such as Alzheimer's disease. In the case of PE, this congophilia can be demonstrated as early as 2 weeks before the onset of symptoms of PE.[8] Thus, facilitating early diagnosis and further management to prevent complications (Table 1).

In this study, we intend to detect misfolded proteins in PE patients by the following two methods: Spectroscopy and Congo red card test in the hope of developing a test that is cheap, non-invasive, and effective in early diagnosis and treatment of PE so that the several dreaded complications of this disease can be prevented in time and the burden of the disease could be reduced (Fig. 1).

[1–4]Department of Obstetrics and Gynaecology, GSVM Medical College, Kanpur, Uttar Pradesh, India

Corresponding Author: Garima Gupta, Department of Obstetrics and Gynaecology, GSVM Medical College, Kanpur, Uttar Pradesh, India, Phone: +91 7985374035, e-mail: amitygarima@gmail.com

How to cite this article: Gupta N, Gupta G, Jahan U, *et al*. Ultraviolet Spectroscopy vs Congo Red Card Test—A Step Forward toward Early Prediction of Preeclampsia: A Case–Control Study. J South Asian Feder Obst Gynae 2024;16(2):192–197.

Source of support: Nil

Conflict of interest: None

Table 1: Characteristics of the cases and controls

Variables	*Case (n = 98)*	*Control (n = 98)*
Maternal age (years) (SD)	26.85	(24.50)
Area (n, %)		
Urban	32 (41.83)	38 (41.83)
Rural	66 (57.14)	60 (57.14)
Education		
Literate	26 (25.51)	27 (25.51)
Illiterate	72 (74.48)	71 (74.48)
Parity		
1	57 (58.16)	59 (60.20)
2	29 (29.59)	19 (19.38)
3+	12 (12.24)	20 (20.40)
Gestational age (completed weeks)		
<34	39 (39.79)	38 (38.77)
34–36	28 (28.57)	20 (20.40)
37–38	18 (18.36)	24 (24.48)
>39	13 (13.26)	16 (16.32)
SBP (mm Hg) at time of diagnosis		
<139	10 (10.20)	98 (100)
140–159	53 (54.08)	0 (0)
>160	35 (35.71)	0 (0)
DBP (mm Hg) at time of diagnosis		
<89	6 (6.12)	98 (100)
90–109	74 (75.51)	0 (0)
>110	18 (18.36)	0 (0)
Vitals		
Stable	90 (91.83)	98 (100)
Unstable	8 (8.16)	0 (0)
LFT		
Normal	55 (56.12)	98 (100)
Abnormal	43 (43.87)	0 (0)
KFT		
Normal	96 (97.95)	98 (100)
Abnormal	2 (2.04)	0 (0)
Platelet count		
Normal	78 (79.59)	98 (100)
Thrombocytopenia (<1 lakh)	20 (20.40)	0 (0)
Proteinuria at the time of diagnosis		
0	0 (0)	98 (100)
1	57 (58.16)	0
2	35 (35.71)	0
3	7 (7.14)	0
Antihypertensive medication		
Yes	7 (7.14)	0 (0)
No	91 (92.85)	98 (100)
Seizure		
No	82 (83.67)	98 (100)
Yes	16 (14.28)	0 (0)
Administered MgSO4		
No	58 (59.18)	98 (100)
Yes	40 (40.81)	0 (0)
Type of PIH		
Eclampsia	16 (16.32)	0 (0)
HELLP	7 (7.14)	0 (0)
PE with severe features	22 (22.44)	0 (0)
PE without severe features	53 (54.08)	0 (0)

DBP, diastolic blood pressure; HELLP, hemolysis, elevated liver enzymes, and low platelets; KFT, kidney function test; LFT, liver function test; PIH, pregnancy induced hypertension; SBP, systolic blood pressure

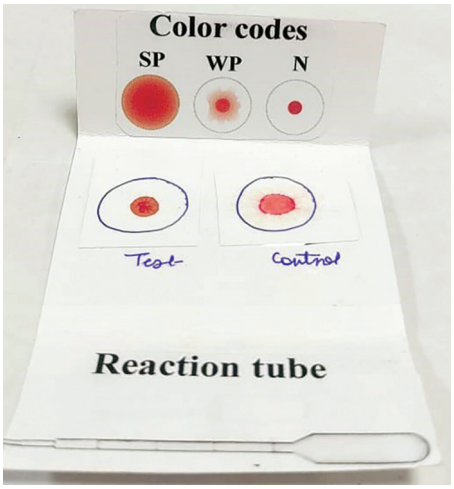


Fig. 1: SP, strongly positive; WP, weakly positive; N, negative

Aim and Objectives

- To establish the role of spectroscopy in the detection of misfolded proteins.
- Comparative evaluation of UV–Vis spectroscopy and Congo red card-based test in the detection of urinary misfolded proteins in PE patients.

Materials and Methods

The study was conducted in the Department of Obstetrics and Gynecology., LLR Hospital, GSVM Medical College, Kanpur, Uttar Pradesh, India from January 2021 to October 2022 on patients who were admitted from the outpatient department (OPD) and emergency (Table 2).

Sample Size

The present study was conducted on 196 study participants including 98 cases and 98 controls [at 95% confidence interval (CI), 80% power of test] after screening them using our inclusion criteria (Fig. 2).

Inclusion Criteria

- All antenatal women of age above 18 years and below 40 years attending OPD or admitted in the hospital between 20 weeks and 41 weeks of gestation age.
- Patients giving consent.

Exclusion Criteria

- Age (<18 years and >40 years),
- Multifetal pregnancy.
- Pregnant women with chronic hypertension and chronic renal failure.
- Pregnant women with comorbidities such as diabetes, epilepsy, kidney diseases, heart diseases, or any other chronic illnesses.

Urine Sample Collection

Freshly obtained mid-stream urine samples for testing misfolded proteins in a sterile container and tested using UV-Vis absorption spectroscopy and Congo red card test.

Assessment of Urine Using Paper-based Congo Red Dye

About 150 µL of fresh urine was mixed with the CRD inserted into the transfer pipet After 1 minute, the mixture was dispensed into roughly equal-sized drops within the printed areas on the paper. At 3 minutes, the result was read. Later, J SOFTWARE was used to calculate pixels of the image of the Congo red stain along with the calculation of the area under it (Fig. 2).

Table 2: Sensitivity and specificity of UV-absorption spectroscopy

UV absorbance	*Sensitivity*	*UV absorbance*	*Specificity*
TP	76	TN	79
FN	22	FP	19
	TP/(TP + FN)		TN/(TN + FP)
	77.53		80.61

FN, false negative; FP, false positive; TN, true negative; TP, true positive

Assessment of Urine Using UV Spectrophotometer

Ultraviolet-visible spectrophotometers (Fig. 3) use a light source to illuminate a sample with light across the UV to the visible wavelength range (typically 190–900 nm). The instruments then measure the light absorbed, transmitted, or reflected by the sample at each wavelength. This information reflects the inherent properties of the sample (Fig. 4).

Statistical Analysis

Mean + standard deviation (SD) and percentage were used for continuous and categorical data respectively. The Chi-square test along with the student's *t*-test was used to determine the differences between categorical variables and continuous variables, respectively; $p < 0.05$ were considered to be statistically significant. The sensitivity and specificity were calculated for each test for diagnosing preeclampsia and compared (Tables 2 and 3).

Results

The results of the UV-absorption spectra scan ranged between 250 and 400 nm is shown in Figure 5. The observations of the spectra curve showed that there was increase in UV-absorption among the cases urine samples compared to the urine samples of the control

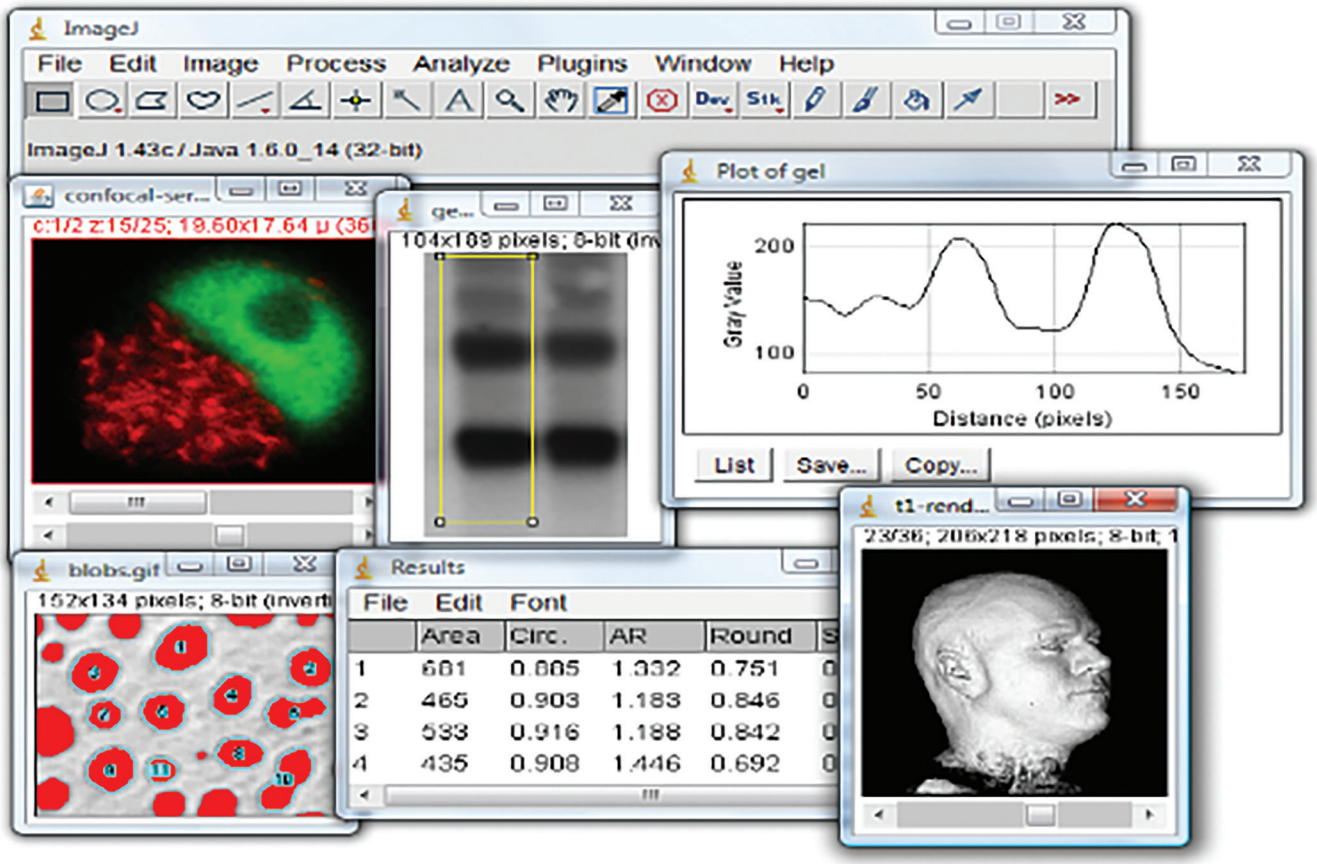


Fig. 2: J software

group. The absorption spectra also showed maximum absorption at 280 nm ƛ as shown in Figure 5B.

The paper-based Congo red dye test result is shown in (Fig. 4), in which the urine sample of cases represented with darker color appearance compared to the control urine samples with a lighter color. The images obtained by the Congo red paper-based test were then processed using IMAGE J software to calculate pixels of the image along with the area covered under the Congo red stain in the paper for both control and case groups.

The results of the Congo red paper-based test in both cases and control showed non-significant differences in pixels of color, in contrast to the area covered by the pixels where the *p*-value was below 0.0001 groups as shown in Tables 4 and 5.

The results of the present study showed that the UV-absorbance method is more sensitive and specific compared to the Congo red paper-based test in detection of the misfolded proteins present in the urine samples of the preeclamptic patients (Table 6).

Unpaired *t*-test was applied for both groups and *p*-value was found to be below 0.0001. Our findings suggest that with increase in severity associated with preeclampsia showed an increase in absorbance in the UV–Vis region and area covered under the Congo red assay that can be implicated in the source of misfolded protein.

Fig. 3: Nanodrop spectrophotometer

Discussion

Previously published studies were conducted to search for a reliable method for diagnosis of preeclampsia using various predictive markers, but such method may have some associated limitations such as a costlier approach, time consuming and cumbersome approach. Our study has compared two methods for the detection of urinary misfolded proteins that can be further used for the diagnosis of preeclampsia.

The present study was conducted on 196 study participants including 98 cases and 98 controls (at 95% CI, 80% power of test). This study aims to assess the sensitivity and specificity of UV-absorbance and Congo red paper-based tests using urine specimens of control and preeclamptic patients.

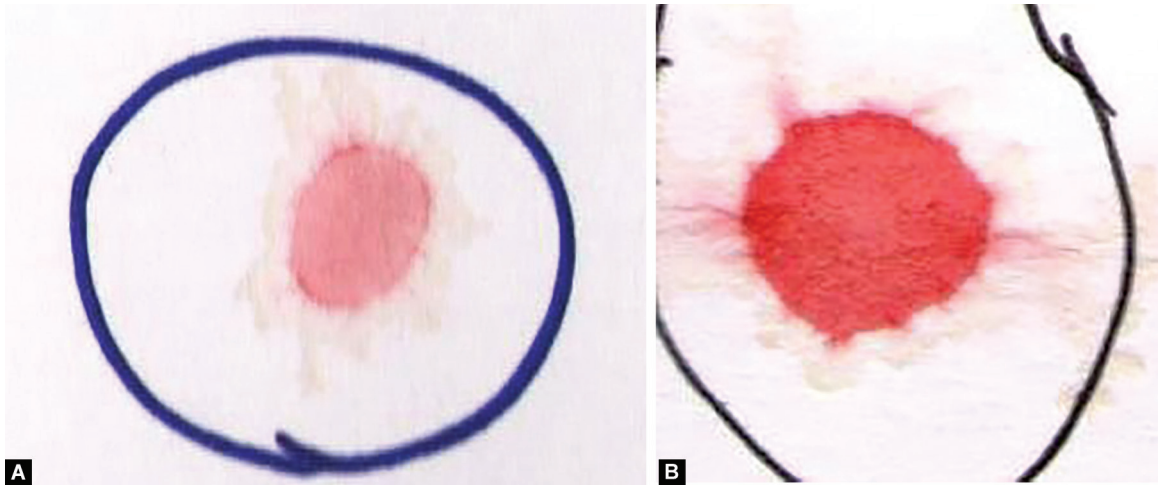
Xing-Min Li et al.[8] developed a point-of-care test device that detects urinary misfolded proteins and compared it to the CRD retention test of Buhimschi et al.[7] and demonstrated it is a useful tool for preeclampsia diagnosis. Their test had sensitivity, 71.62%; specificity, 97.11%; PPV, 72.60%; and NPV, 96.97%. Better performance was found in early-onset preeclampsia, with sensitivity, 85.71%; specificity, 96.96%; PPV, 85.71%; and NPV 96.96%, which is comparable to our results in the Indian rural population.

In another study, Rood et al. concluded CRD paper test was positive in 25% of the cases and that it outperformed measured serum and urine markers (80.2% sensitivity, 89.2% specificity, 92.1% negative predictive value, 86.7% accuracy).[9] Nagarajappa et al. concluded that factors such as GA of onset, severity, a superimposition by eclampsia, fetal growth restriction, or stillbirth

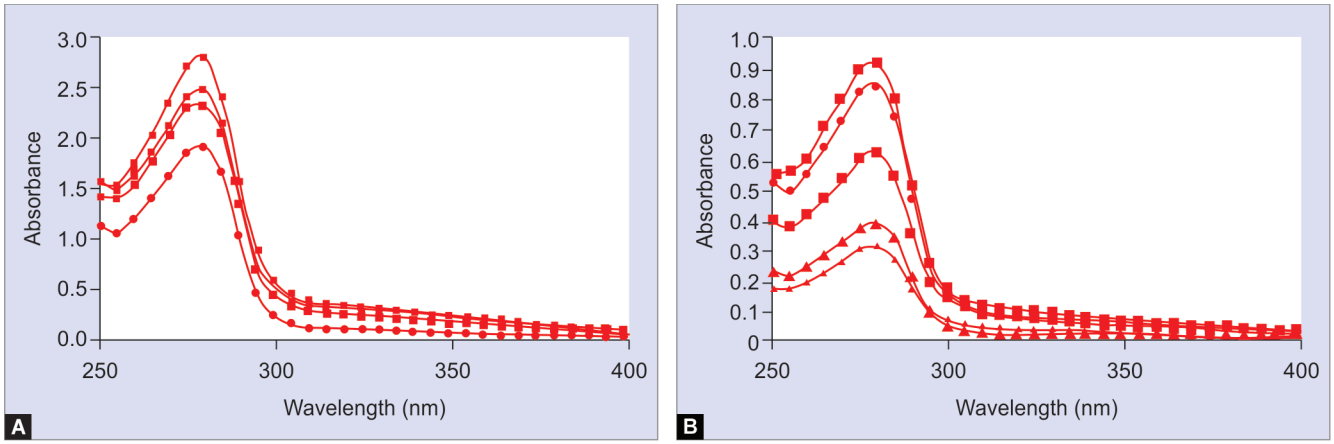
Table 3: Sensitivity and specificity of Congo red paper-based test

Congo red	*Sensitivity*	*Congo red*	*Specificity*
TP	69	TN	63
FN	29	FP	35
	TP/(TP + FN)		TN/(TN + FP)
	70.40		64.28

FN, false negative; FP, false positive; TN, true negative; TP, true positive



Figs 4A and B: (A) Control figure; (B) Cases



Figs 5A and B: (A) Controls; (B) Cases

Table 4: Characteristics of pixels and area under Congo red image in control vs cases

	Control	*Cases*	*p-value*
Pixels of Congo red	211.92 ± 15.32	216.53 ± 18.85	0.618
Area under Congo red	10146.48 ± 211.22	16653.61 ± 128.66	<0.0001*

*Significant of *p*-value

Table 5: Comparison of sensitivity and specificity of UV-absorption spectroscopy and Congo red paper-based test

	Sensitivity	*Specificity*	*Positive predictive value*	*Negative predictive value*	*Positive likelihood ratio*	*Negative likelihood ratio*	*Accuracy (%)*
UV absorbance	77.53	80.61	80	78.21	4	3.5	79.08
Congo red	70.40	64.20	66.34	68.47	1.97	0.46	67.3

Table 6: Comparison between severity of the preeclampsia with the UV-absorbance and Congo red area

Type of PIH	*UV absorbance N (mean ± SD)*	*Congo red area N (mean ± SD)*
Preeclampsia + severe features	22 (2.771 ± 0.623)	22 (14338.06 ± 53.96)
Preeclampsia + severe features	53 (2.039 ± 0.102)	53 (9485.33 ± 56.23)
Eclampsia	16 (1.763 ± 0.136)	16 (9234.57 ± 49.56)
HELLP	7 (1.549 ± 0.234)	7 (8718.22 ± 67.24)

HELLP, hemolysis, elevated liver enzymes, and low platelets; PIH, pregnancy induced hypertension

had no effect on urinary congophilia.[10] However, our study shows that when the area of the image is compared a significant increase is seen when preeclampsia has added features of severity. Anjali Rani et al. in her study found that 30 out of 250 patients with positive Congo red test develop preeclampsia, so the chance of developing preeclampsia was 12% with PPV 58.8, NPV 95.4%, sensitivity 66.7%, specificity 93.6%, and accuracy 90.4%.[11]

Proteins that have been misfolded or denatured can form associations in solution, the formation of insoluble aggregates, and also are structurally different from naturally folded proteins. The results of our study showed maximum absorption at 280 nm for the urine samples of cases compared to the control samples. This maximum absorption might be due to structural changes in the protein conformation and aggregates formation, which increases the exposure of aromatic and side chain amino acids toward the outer microenvironment so that it contributes in maximum absorption. This study has revealed an unprecedented and novel use of spectroscopy in the field of obstetrics. Our results demonstrate sensitivity, 77.53; specificity, 80.61; PPV, 80; NPV, 78.21; and accuracy 79.08% with this test which is superior to several Congo red tests done in previous studies.

On comparing the results of both techniques, it was found that UV-absorption spectroscopy is more sensitive and specific compared to Congo red paper-based test because it depends on the absorption of each aromatic and side chain amino acids that is the major limitation in CRD, which gives only staining with aggregates of protein only using calorimetric approach.

Our findings suggest that with increase in severity associated with preeclampsia showed an increase in absorbance in UV-Vis region and area covered under Congo red assay that can be implicated into the source of misfolded protein. This study confirms that urinary congophilia and UV absorbance level is elevated in preeclamptic women compared to normotensive pregnant women in the Indian population.

Limitations of the Study

- Smaller sample size.
- Prospective cohort could be a better study design.

Strengths of the Study

- One of the few studies done in the rural population of North India.
- Use of spectroscopy in this context is novel and groundbreaking.

- Spectroscopy is found to be better in terms of statistical results and comparable in terms of cost, speed, and non-invasiveness.
- Our findings have added more information in the limited knowledge on this subject.

Conclusion

Preeclampsia is a major problem nowadays and is associated with adverse pregnancy outcomes. Early detection of preeclampsia needs some simple, non-invasive, express, affordable methods using body secretion for its diagnosis. Timely screening of the preeclampsia can help the patients in timely referral to the higher center for effective management. The Congo red assay is a calorimetric qualitative assay based on the detection of urinary misfolded protein and has been proven to be a good choice to detect preeclampsia within the community. However, our In-house UV–Vis absorption spectroscopy is a unique and novel test for diagnosing misfolded proteins in the urine of preeclamptic patients. It has proved to be a more specific and sensitive method for the detection of urinary misfolded proteins compared to the Congo red assay and can be a breakthrough in the diagnosis of preeclampsia.

Orcid

Garima Gupta https://orcid.org/0000-0001-5392-2015
Sonali Bisht https://orcid.org/0000-0002-1454-1268

References

1. World Health Organization. The World Health Report 2005. Make every mother and child count. Available at: https://www.who.int/publications/i/item/9241562900. Accessed on: 30 January 2020.
2. Duhig K, Vandermolen B, Shennan A. Recent advances in the diagnosis and management of pre-eclampsia. F1000Res 2018;7:242. DOI: 10.12688/f1000research.12249.1.
3. Wiktor H, Kankofer M, Schmerold I, et al. Oxidative DNA damage in placentas from normal and pre-eclamptic pregnancies. Virchows Archiv 2004;445(1):74–78. DOI: 10.1007/s00428-004-1024-2.
4. Serdar Z, Gür E, Colakoethullarý M, et al. Lipid and protein oxidation and antioxidant function in women with mild and severe preeclampsia. Arch Gynecol Obstet 2003;268(1):19–25. DOI: 10.1007/s00404-002-0302-y.
5. Buhimschi IA, Zhao G, Funai EF, et al. Proteomic profiling of urine identifies specific fragments of SERPINA1 and albumin as biomarkers of preeclampsia. Am J Obstet Gynecol 2008;199(5):551.e1–e16. DOI: 10.1016/j.ajog.2008.07.006.
6. Díaz–Villanueva J, Díaz–Molina R, García–González V. Protein Folding and Mechanisms of Proteostasis. Int J Mol Sci 2015;16(8):17193–17230. DOI: 10.3390/ijms160817193.
7. Buhimschi IA, Nayeri UA, Zhao G, et al. Protein misfolding, congophilia, oligomerization, and defective amyloid processing in preeclampsia. Sci Transl Med 2014;6(245):245ra92. DOI: 10.1126/scitranslmed.3008808.
8. Li X-M, Liu X-M, Xu J, et al. Late pregnancy screening for preeclampsia with a urinary point-of-care test for misfolded proteins. Spradley FT, editor. PLoS One 2020;15(5):e0233214. DOI: 10.1371/journal.pone.0233214.
9. Rood KM, Buhimschi CS, Dible T, et al. Congo red dot paper test for antenatal triage and rapid identification of preeclampsia. EClinicalMedicine 2019;8:47–56. DOI: 10.1016/j.eclinm.2019.02.004.
10. Nagarajappa C, Rangappa SS, Suryanarayana R, et al. Urinary congophilia in preeclampsia: Experience from a rural tertiary-care hospital in India. Pregnancy Hypertens 2018;13:83–86. DOI: 10.1016/j.preghy.2018.05.006.
11. Rani A, Jain M, Dash D, et al. A study on the role of urinary congophilia in early detection of preeclampsia. Ann Natl Acad Med Sci 2022;58(02):087–091. DOI: 10.1055/s-0042-1743134.