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Short Communication

A Novel Diagnostic Test for Pre-Eclampsia: Congo Red Dot Test

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ABSTRACT

Preeclampsia(PE) is a disorder of pregnancy characterized with hypertension (after week 20), proteinuria and affected systemic systems. Preeclampsia is a leading cause of maternal mortality worldwide and it is of importance to come up with economically reasonable biomarkers to predict it. Thus, Urine congophillia in PE has been studied. Misfolded proteins are detected in the urine and serum of such patients. These proteins bind easily to various dyes especially Congo red dye, which is the basis of this diagnostic test. The article will highlight the pathology of preeclampsia, type of protein in the urine of preeclamptic patients and Congo red test for preeclampsia.

Key Words: Preeclampsia, Congo Red Dye, Congophilia, Proteinuria.

Introduction

Pre-eclampsia (PE) is disorder associated with pregnancy. It is defined as a disorder of hypertension and proteinuria in pregnant women.[1, 2] PE is the arterial hypertension spotted after 20th week of pregnancy estimated to occur in 3-5% of pregnancies. It imparts a risk of maternal and fetal morbidity and mortality if not managed properly.[1,2,3] It accounts for approximately 70, 000 maternal deaths and 500 ,000 fetal deaths worldwide every year.[1] Pre-eclampsia is a multisystem and life threatening disorder that involves proteinuria and affects the maternal kidneys, liver, brain, clotting systems and primarily, the placenta. These are enlisted in Table 1.[3]

Table 1Maternal organ dysfunctions in preeclampsia

Maternal Organ Dysfunctions in PE
Renal function loss
Hepatic dysfunction
Neurological complications
Hematological dysfunction

This article aims to present the use of proteinuria for the timely diagnosis of preeclampsia. The method employed is the use of dye Congo red. The Information was extracted from PubMed and Google Scholar searches using keywords as: "preeclampsia", "congophilia", and "Congo red dye".

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Pathology of Preeclampsia

The hypertensive disorders of pregnancy are classified according to time period. Hypertension diagnosed before week 20 of gestation is known as chronic hypertension and that diagnosed after 20 weeks is called gestational hypertension or preeclampsia, as shown in figure 1.[4,5]

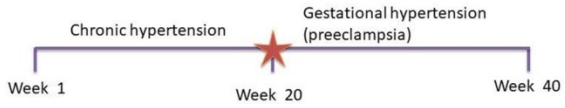

Figure 2: Hypertension in pregnancy

Preeclampsia is classically defined as hypertension with proteinuria that develops in second trimester of pregnancy.[1] Preeclampsia can persist after delivery, and in some cases, develop de novo postpartum.[6] In 2013, a revised definition for preeclampsia suggested that the severe features may be present with or without proteinuria.[7] Many clinical studies have determined placenta as the center of its pathogenesis.[8] Preeclampsia progresses in two stages; abnormal placentation early in first trimester followed by a maternal preeclamptic syndrome in late second and third trimesters.[9]This second phase is marked with increased levels of anti-angiogenic factors causing a hypertensive, multi-organ failure response.[10] Defective remodeling of uterine spiral arteries due to immunological response at maternal-fetal interface results in ischemic placenta.[11] Failing stressed ischemic placenta causes an over secretion of anti-angiogenic factors like soluble fms-like tyrosine kinase 1(sFlt-1)

and soluble Endoglin (sEng) which are responsible for maternal hypertension and proteinuria.[12] Placental ischemia is also associated with reduced levels of endothelial nitric oxide (e-NOS), a vasodilator, and heme oxygenase-2, an antioxidant, resulting in more oxidative stress and formation of micro emboli.[13,14] Toll-like receptor 4 (TLR4), responsible for induction of inflammatory cytokines, are over-expressed in placenta and kidneys. This leading to HELP (Hemolysis, Elevated Liver enzyme, and low Platelets) syndrome of preeclampsia.[15]

Major risk factors for preeclampsia are described in (table 2).

Table-2: Risk Factors for preeclampsia

Risk Factors For PE
Prior preeclampsia
Multiple gestation
Systemic lupus erythematosus
Chronic hypertension
Diabetes mellitus

Pre-eclampsia and Associated Proteinuria:

PE patient's urine analysis presents a characteristic misfolding of proteins known as Congophilia, which contributes to the severity of this disease.[16] Pathophysiology of hypertension includes free radical activity, oxidative stress, and increased reactive oxygen species. These reactive species targets proteins in particular, which leads to the aggregation of misfolded proteins, the amyloids.[17]

Kidney damage is a precursor of proteinuria. The glomerular filtration barrier consists of endothelium, basement membrane and podocyte. This barrier is damaged by an imbalance in angiogenic proteins leading to podocyte and endothelial injury.[18,19] Endothelial vacuolization is also found to be linked to preeclampsia.[20]

In preeclamptic pregnancies, the glomerular filtration rate and the renal plasma flow is reduced, and this alters the glomerular permeability which causes proteinuria. Misfolded proteins are also detected in the serum and placenta of Preeclamptic patient.[17] They can be cytotoxic and can contribute to the neurological disorders including Parkinson's and Alzheimer's disease.[16, 17, 21]

Urine analysis of preeclampsia patient (PE) is crucial because proteinuria is a commonly presenting symptom of preeclampsia patients. Urine analysis of PE patients is done by various methods. Visual reagent strip tests, the total protein estimation in a 24-hour urine sample and the spot urine protein: creatinine ratio are some methods used to access severity of pre-eclampsia by taking urine sample and using urine dipsticks to analyze its characteristics.[22] After 20th week of pregnancy, PE is detected if the proteinuria is greater or equal to 300mg in a 24-hour collection, the urine protein: creatinine ratio is 0.3mg/dl or if the urine dipstick shows a value of +1.[23,21]

The primary excretion of urinary proteins include albumin, alpha 1 and alpha 2 globulin, beta globulin, gamma globulin, ceruloplasmin, pseudo-cholinesterase and alpha 2 macroglobulin [23,17] These patients have also shown an increased mRNA levels for secretases that cleave Amyloid Precursor Protein (APP) to yield A beta. Proteins that are capable of Amyloid aggregation in preeclampsia are presented in TABLE 3.[17]

Table-2: Proteins that Exhibit Amyloid Aggregation

Protein Exhibiting Amyloid Aggregation
Amyloid beta protein
Alpha-1 antitrypsin (AAT)
Light chains of immunoglobulins
Transthyretin

Amyloid β protein (aβ) in PE:

Amyloid β protein accumulation contributes to preeclampsia. Alzheimer's β amyloid and other amyloid-like proteins have been observed in urine of PE patients.[21]Researchers found that amyloid aggregation can cause improper folding and aggregation, and this aggregation can even cause placental ischemia.[21]

alpha-1 antitrypsin (AAT) in PE:

It is a serine protease inhibitor present in plasma. It is aggregated and misfolded in response to conditions like oxidative stress.[17] In preeclamptic women, the aggregation and misfolding of AAT is a manifestation of PE.[17]

Light chains of immunoglobulins in PE:

Preeclamptic pregnancies can present with abnormal immunoglobulins (aggregation of misfolded light chains) in the urine. [17] Its aggregation has also contributed to pathogenicity of amyloidosis and multiple myeloma.[17]

Transthyretin in pe:
Transthyretin is a protein. It acts as a transporter of thyroxine and retinol. In preeclampsia, it is seen to be dysregulated and aggregated. This lead to oxidative stress and defective deep placentation, thus contributing to PE.[17]

Protective role of human pregnancy zone proteins:
Some Extracellular proteins named chaperons (including caseins, clusterins, haptoglobin) inhibit and prevent protein misfolding and aggregation. Normally, in pregnant woman chaperons are present in high amount preventing the misfolding of proteins. In case of decreased production of PZP or their dysregulation the proteins undergo aggregation leading to PE.[17]

Congo red test for preeclampsia
Congo red dye was synthesized as a direct dye by Bottiger, who was in an attempt to create a pH indicator.[24] It was latter used for tissue staining, testing acid in intestinal tract of animal and diagnosing amyloid fibrils.[24] Congo red is chemically a sodium salt of benzidinediazo-bis-1-naphtylamine-4-sulphonic acid.[26] Congo red binds to amyloid by non-ionic intereaction.[27]
The presence of amyloid fibril in urine of preeclamptic women and the ability of Congo red to bind to amyloid fibril, has led to several clinical trials for the assessing of the utility of this dye to diagnose pre-eclampsia. The test is named Congo-Red Dot (CRD) test. These studies are mentioned in table 4.

Table-3: Clinical trials of Preeclampsia based on Congo Red Dot test(https://clinicaltrials.gov)

Title of Study	Study Design	Enrollments	Recruitment Status
Diagnosis and Prediction of Pre-Eclampsia by Using Congo Red Dot Test in Bangladesh and Mexico NCT (02381210)	Case-control	400	Completed
The Clinical Utility of the Congo-Red Dot Test for Diagnosis and Early Prediction of Preeclampsia During Pregnancy NCT (02455544)	Cohort	346	Completed
Assessing the Usability and Clinical Utility of the Congo Red Dot Test: A Survey NCT (02611011)	Other	198	Completed
Assessing the Usability and Clinical Utility of the Congo Red Dot Test: A Case-control Study NCT (02610972)	Case-control	150	Completed

Urinary congophillia is important in grouping normotensive pregnant women form preeclamptic women various methods have been employed to determine the efficiency of this test. This includes Cap Cord test, GV-005(beta prototype lateral flow diagnostic device) and High man's method.[28, 29, 30]
The device for Cap Cord test consists of a pipette, Congo red dye and an applicator.[28] The applicator is used to spread mixture of urine and Congo red on the cellulose membrane. The misfolded protein binds to Congo red and forms different patterns on the cellulose membrane. These dyeing patterns are classified into six categories. This method depicted that detection of preeclampsia was significant in the tested preterm cases.[28]
The other diagnostic device used for preeclampsia was GV-005. This study was performed in Bangladesh and Mexico. Results showed that it is an important method for rapid detection of preeclampsia .[29]
The High man's method uses alcoholic solution for staining. This method was unique from the ones previously mentioned because the urine was centrifuged, thus urinary sediment was employed in this method. This study confirmed that Congo red is a promising diagnostic test for preeclampsia due to the specificity of this dye for misfolded protein.[30]
There has been development of Smartphone-based diagnostic test for this disease with the objective to provide early detection of the condition so that mortality rate can be reduced, especially in regions which have limited resources.[30]

Discussion

Hypertension in pregnancy can be life threatening. Preeclampsia is one of the disorders which leads to damage to various systems of the mother such as liver and kidney. Preeclampsia is one of the leading causes of maternal mortality worldwide and it is of importance to come up with economically reasonable biomarkers to predict it. Thus, Urine congophillia in PE has been studied.
The key feature of PE is its association with proteinuria. Protein in urine is abnormal protein or misfolded proteins. Some of the primary proteins are albumin, alpha 1 and alpha 2 globulin, beta globulin, gamma globulin, ceruloplasmin, pseudo-cholinesterase and alpha 2 macroglobulin. The misfolded protein form amyloid fibrils. Presence of such proteins in urine and the ability of such protein to bind to Congo red dye has been a remarkable finding as it can be utilized to diagnose PE.

In routine settings, the diagnosis is done by renal function test, liver function test, blood test and Doppler test. The protein concentration is observed in urine. ALT and AST levels are detected in liver function test. Increased hematocrit is seen in blood test. In uterine artery doppler, increased resistance and slow flow is detected. Some of these methods require hours whereas Congo red dot test just requires 3-4 minutes. This efficiency in time is significant. This method is non-invasive as compared to taking a blood sample.

The most important feature of this test is that it does not require an elaborate set of equipment and laboratory professionals. It can be performed by the mother without any assistance. Thus, it can be a leading substitute and an alternative for the current diagnostic methods. The test can lead to a better health outcome because of its cost efficacy and ease of use. Women in remote areas would be able to detect PE easily where laboratory facilities are not well developed. This article has elaborated the clinical trials done to confirm the accuracy of the test and the various method used. Each method in the trials is unique but each one has shown a positive result.

It can be concluded that Congo red dot test for preeclampsia seems to be a promising tool to fight the high morbidity and mortality of preeclampsia. This will help to increase the survival rate of patients with preeclampsia because the cost of the test needed to confirm PE will decrease significantly. This will help in an early diagnosis which will lead to an effective management of the disease. It also highlights the necessity for further research so that this test can be made easily available and feasible for every pregnant woman to rule out the possibility of hypertension

References

1. Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: pathophysiology, challenges, and perspectives. Circulation research. 2019 Mar 29;124(7):1094-112.
2. .Ramos JGL, Sass N, Costa SHM. Preeclampsia. Rev Bras Ginecol Obstet. 2017 Sep;39(9):496-512. English. doi: 10.1055/s-0037-1604471. Epub 2017 Aug 9
3. El-Sayed AA. Preeclampsia: A review of the pathogenesis and possible management strategies based on its pathophysiological derangements. Taiwanese Journal of Obstetrics and Gynecology. 2017 Oct 1;56(5):593-8.
4. Pillay P, Moodley K, Moodley J, et al. Placenta-derived exosomes: potential biomarkers of preeclampsia. International Journal of Nanomedicine. 2017;12:8009.
5. Redman CW. Early and late onset preeclampsia: Two sides of the same coin. Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health. 2017 Jan 1;7:58.
6. Goel A, Maski MR, Bajracharya S, et al. Epidemiology and mechanisms of de novo and persistent hypertension in the postpartum period.Circulation. 2015; 132:1726–1733.
7. ACOG practice bulletin no. 202 summary: gestational hypertension and preeclampsia.Obstet Gynecol. 2019; 133:211–214.
8. Roberts JM, Hubel CA: The two stage model of preeclampsia: Variations on the theme. Placenta 30[Suppl A]: S32–S37, 2009
9. Romero R, Chaiworapongsa T. Preeclampsia: a link between trophoblast dysregulation and an antiangiogenic state.J Clin Invest. 2013; 123:2775–2777. doi: 10.1172/JCI70431
10. Palei AC, Spradley FT, Warrington JP, et al. Pathophysiology of hypertension in pre-eclampsia: a lesson in integrative physiology.Acta Physiol (Oxf). 2013; 208:224–233. doi: 10.1111/apha.12106
11. N. Harihana, A. Shoemker, S. Wagner.Pathophysiology of hypertension in preeclampsia Clin Pract, 13 (2016)
12. S.E. Maynard, J.Y. Min, J. Merchan, et al.Excess placental soluble fms-like tyrosine kinase I (sFlt-1) may contribute to endothelial dysfunction, hypertension and proteinuria in pregnancyJ Clin Investig, 111 (5) (2003)
13. F. Li, J.R. Hagamon, H. Kim, et al.eNOS deficiency acts through endothelia to aggravate sFlt-1 induced preeclamptic-like phenotype J Am Soc Nephrol, 23 (4) (2012)
14. Lyall, A. Barber, L. Myalt, et al. Heme-oxygenase expression in human placenta and placental bed implies a role in regulation of trophoblast invasion and placental function FASEB J, 14 (1) (2000)
15. G.V. Kulikova, N.V. Nizyaeva, M.N. Nagovitsina, et al.Specific features of TLR4 expression in structural elements of placenta in patients with preeclampsia Placenta, 43 (2016)
16. Millen KR, Buhimschi CS, Zhao G, et al.. Serum and Urine Thioflavin-T-Enhanced Fluorescence in Severe Preeclampsia. Hypertension. Epub 2018 Apr 23. PMID: 29686018; PMCID: PMC5945331. 2018 Jun;71(6):1185-1192. doi: 10.1161/HYPERTENSIONAHA.118.11034.
17. Gerasimova EM, Fedotov SA, Kachkin DV, et al. Protein Misfolding during Pregnancy: New Approaches to Preeclampsia Diagnostics. Int J Mol Sci. 2019;20(24):6183.
18. Brownfoot FC, Hannan NJ, Cannon P, Nguyen V, Hastie R, Parry LJ, Senadheera S, Tuohey L, Tong S, Tu'uhevaha J. Sulfasalazine reduces placental secretion of antiangiogenic factors, up-regulates the secretion of placental growth factor and rescues endothelial dysfunction. EBioMedicine. 2019 Mar 1;41:636-48.
19. Turner RJ, Bloemenkamp KW, Penning ME, Bruijn JA, Baelde HJ. From glomerular endothelium to podocyte

pathobiology in preeclampsia: a paradigm shift. Current hypertension reports. 2015 Jul;17(7):1-8.
20. Gaber LW, Spargo BH, Lindheimer MD. 12 Renal pathology in pre-eclampsia. Bailliere's clinical obstetrics and gynaecology. 1994 Jun 1;8(2):443-68.
21. Do JP, Cao KJ, Wei S, et al. Identification of Patients with Preeclampsia by Measuring Fluorescence of an Amyloid-Binding Aryl Cyano Amide in Human Urine Samples. Anal Chem. 2018 Dec 18;90(24):14316-14320.
22. . Kattah A, Milic N, White W, et al. Spot urine protein measurements in normotensive pregnancies, pregnancies with isolated proteinuria and preeclampsia. Am J Physiol Regul Integr Comp Physiol. 2017;313(4):R418-R424.
23. Phipps EA, Thadhani R, Benzing T, Karumanchi SA. Pre-eclampsia: pathogenesis, novel diagnostics and therapies [published correction appears in Nat Rev Nephrol. 2019 Jun;15(6):386]. Nat Rev Nephrol. 2019;15(5):275-289.
24. Steensma DP. "Congo" red: out of Africa?. Arch Pathol Lab Med. 2001;125(2):250-252. doi:10.1043/0003-
25. Yakupova EI, Bobyleva LG, Vikhlyantsev IM, et al. Congo Red and amyloids: history and relationship. Bioscience reports. 2019 Jan 31;39(1).
26. Puchtler H, Sweat F, Levine M. On the binding of Congo red by amyloid. Journal of Histochemistry & Cytochemistry. 1962 May;10(3):355-64.
27. Nagarajappa C, Rangappa SS, Suryanarayana R, et al. Urinary congophilia in preeclampsia: experience from a rural tertiary-care hospital in India. Pregnancy hypertension. 2018 Jul 1; 13:83-6.
28. Li XM, Liu XM, Xu J, Du J, et al. Late pregnancy screening for preeclampsia with a urinary point-of-care test for misfolded proteins. Plos one. 2020 May 20; 15(5):e0233214.
29. Bracken H, Buhimschi IA, Rahman A et al. Congo red test for identification of preeclampsia: Results of a prospective diagnostic case-control study in Bangladesh and Mexico. EClinicalMedicine. 2020 Dec 22; 31:100678.
30. Rodríguez Chávez JL, Fuentes Gutiérrez EK, Angeles Vázquez MD, et al. Evaluation of Congo red staining kit to determine proteinuria in preeclampsia.
31. Jonas SM, Deserno TM, Buhimschi CS, et al. Smartphone-based diagnostic for preeclampsia: an mHealth solution for administering the Congo Red Dot (CRD) test in settings with limited resources. Journal of the American Medical Informatics Association. 2016 Jan 1;23(1):166-73.