International Journal of
Health Sciences
(IJHS)

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CARI Journals

International Journal of Health Sciences

Vol. 5, Issue No. 1, pp 8 - 16, 2022

www.carijournals.org

LIPID PEROXIDATION AND EXPRESSION OF PARAOXONASE-1 (PON-1) GENE IN PATIENTS WITH PREECLAMPSIA

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ABSTRACT

Purpose: To evaluate lipid peroxidation and expression of paraoxonase-1 (pon-1) gene in patients with preeclampsia.

Methodology: The study comprised of 160 participants, in which 80 were normotensive pregnant women (Group A) and 80 were preeclamptic pregnant women (Group B). Serum MDA levels and PON-1 gene activity were estimated. The expression of data was subjected to statistical analysis using SPSS version 20.0. One-way analysis of variance (ANOVA) was used to compare the test samples, p value <0.05 was considered significant.

Findings: MDA levels were significantly elevated in preeclamptic pregnant women when compared to normotensive. Also, PON-1 gene expression levels were significantly decreased in preeclamptic pregnant women when compared to normotensive.

***Unique contribution to theory, practice and policy*:** The significant increase in the plasma level of Malondialdehyde and decreases expression of PON-1 gene could be responsible for the presence of oxidative stress and its associated complications in PE patients.

Keyword: *Preeclampsia, Lipid Peroxidation, Paraoxonase-1 Oxidative Stress, Malondialdehyde,*

INTRODUCTION

Pre-eclampsia (PE) is a major disease of human pregnancy, marked by hypertension and proteinuria, appearing during the second or third trimester of gestation. Incidence differs depending on geographical region, time of year, nutrition, race/ethnicity, and affects roughly 3–8% of women worldwide (Steegers *et al.*, 2010, Zabul *et al.*, 2015). PE can be described as maternal hypertension (>140/90 mm Hg systolic/diastolic blood pressure) and proteinuria (>300 mg/24 h). In more serious cases, comorbidities such as hepatic alterations (HELLP syndrome), edema, disseminated vascular coagulation (DIC), and eclampsia may be developed in the pregnant women, particularly cerebral edema. The complications experienced by the fetus as a result of PE include growth restriction leading to low birth weight (1/3 of cases), prematurity, and fetal death. From the onset of symptoms, the disease progressively worsens, potentially leading to both fetal and maternal mortality. It most times requires premature labor induction in severe cases with risks of premature neonates and no cure has been in place for it.

Pre-eclampsia (PE) and its complications and associated pathologies have become one of the main causes of maternal and fetal morbidity and mortality in the world, causing nearly 40% of births delivered before 35 weeks of gestation. Moreover, PE has been strongly associated with an increased risk of later-life death due to cardiovascular disease, independent of other risk factors (Bellamy *et al.*, 2007). The understanding of the underlying factors that explain the pathogenesis of PE and the early identification of the patients at risk of the disease will help in the development of preventative or early therapeutic interventions, aimed to reduce the associated morbidity and mortality during pregnancy, but also the long-term severe problems that PE may produce or is associated with.

Majority of PE is characterized with abnormal placental implantation that defines its severity and gestational gene of presentation. The severity is however induced by the degree of inflammatory response as a result of increased oxidative stress in maternal circulation. The pathogenesis of PE is still not clear yet but the occurrence of PE within families is opening a possibility of genetic link to the pathogenesis of this disorder. Gene associated with generation or inactivation of reactive oxygen species and lipid peroxidation could be plausible and possibly has a link with the etiology of PE.

Lipid peroxidation can be described generally as a process under which oxidants such as free radicals or nonradical species attack lipids containing carbon-carbon double bond(s), especially polyunsaturated fatty acids (PUFAs) that involve hydrogen abstraction from a carbon, with oxygen insertion resulting in lipid peroxyl radicals and hydroperoxides as described previously (Yin *et al.*, 2011). Glycolipids, phospholipids, and cholesterol are also well-known targets of damaging and potentially lethal peroxidative modification. As with any radical reaction, the reaction consists of three major steps: initiation, propagation, and termination. The chemical products of this oxidation are known as lipid peroxides or lipid oxidation products (LOPs) (Ho *et al.*, 2010).

Paraoxonase (PON1) belongs to a family of three serum paraoxonases, including PON2 and PON3; however, PON1 remains the most popular member of this family (Précourt

et al., 2011). PON1 is a calcium-dependent enzyme consisting of 354 amino acids with a molecular mass of 43 kDa. Structural analysis using X-ray crystallography revealed the six-bladed β-propeller structure of PON1, with a central tunnel that houses two calcium ions.

PON1 is a serum esterase synthesized in the liver. The enzyme was originally found to be responsible for the hydrolysis of paraoxone, a toxin that irreversibly inhibits acetyl cholinesterase (Babacan *et al*., 2011). In the circulation, PON1 is bound to high density lipoprotein (HDL). Some of the anti-oxidative and antiinflammatory actions of HDL are attributed to PON1 (Laivuori *et al*., 2006). PON1 is strictly dependent on calcium for its enzymatic activity (Babacan *et al*., 2011).

Methodology

A case control study research design was adopted for the study.

Inclusion Criteria for tests and Control

Participant diagnosed to have Pre-eclampsia as defined by the blood pressure were enrolled in the study. One hundred and sixty pregnant women, within the age range of 18–40 years, in their second and third trimester and with blood pressure >140/90 mmHg will be selected for the study. Normal pregnant women, diagnosed on clinical and ultrasonography findings will be taken as controls.

Exclusion Criteria for Cases and Control

Pregnant women with family history of diabetes in first-degree relatives, Preeclampsia in previous pregnancy and occurrence of any form of pre-pregnancy diabetes were not used for the study. Subjects will also have to be non-smokers, non-alcoholics, and not suffering from any acute infections or chronic illnesses apart from pre-eclampsia.

Subjects

Subjects were recruited from Wesley Guild Hospital, Ilesa, Osun state, Nigeria. One hundred and Sixty participants were used for the study, aged between the 18 and 40 and they were grouped into two:

GROUP A - 80 Non-preeclamptic pregnant women (Control group)

GROUP B – 80 Pre-eclamptic pregnant women

The subjects diagnosed to have Pre-eclampsia as defined by the blood pressure (>140/90 mmHg) were enrolled in the study. Normal pregnant women, diagnosed on clinical and ultrasonography findings will be taken as controls.

Ethical Clearance

This study was approved by the research ethics committee of Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria with ethical clearance code of ORD/AD/EAC/19/087.

Collection of blood samples

Blood samples of all 160 participants were randomly taken starting from 24th week. 5ml of fasting venous blood was collected under aseptic precautions into serum vacutainers. Blood was allowed to stand at room temperature for two hours and then centrifuged at 1500 x g at 4 °C for 15 minutes to harvest serum. Serum was stored in Eppendorf tubes at - 20^0C for further serum biochemical analysis.

For gene expression analysis, 2 milliliters of the blood will be dispensed into plain vacutioner and 2ml DNA/RNA shield will be added to the plasma in order to preserve the DNA/RNA rupturing and the blood from clothing. Then the samples will be stored at room temperature until gene test are performed.

Biochemical Analysis

The total oxidant status (TOS) of serum was measured using an automated colorimetric measurement method developed by Erel (2005) (Rel Assay Diagnostics).

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) present in the test sample which is produced during lipid peroxidation according to the method of Varshney and kale (1990).

Determination of PON-1 gene expression in both patients and control subjects was carried out according to the method of DNA extraction kits and agarose gel electrophoresis as described by Blanca *et al.*, (2014)

Table 1: Sequence for Real Time Quantitative PCR

GENE	FORWARD PRIMERS	REVERSE PRIMERS
PON-1	CAGGAACCACCAGTCTTCTTAC	GATACTGCCTAATGGACTGGC

Statistical Analysis

The distribution of the analyzed expression data from gel images was subjected to statistical analysis using SPSS version 20.0. The result obtained were grouped and expressed as Mean ± Standard error of Mean. One-way analysis of variance (ANOVA) was used to compare the test samples, p value <0.05 was considered significant.

RESULT

Malondialdehyde (MDA) Activity

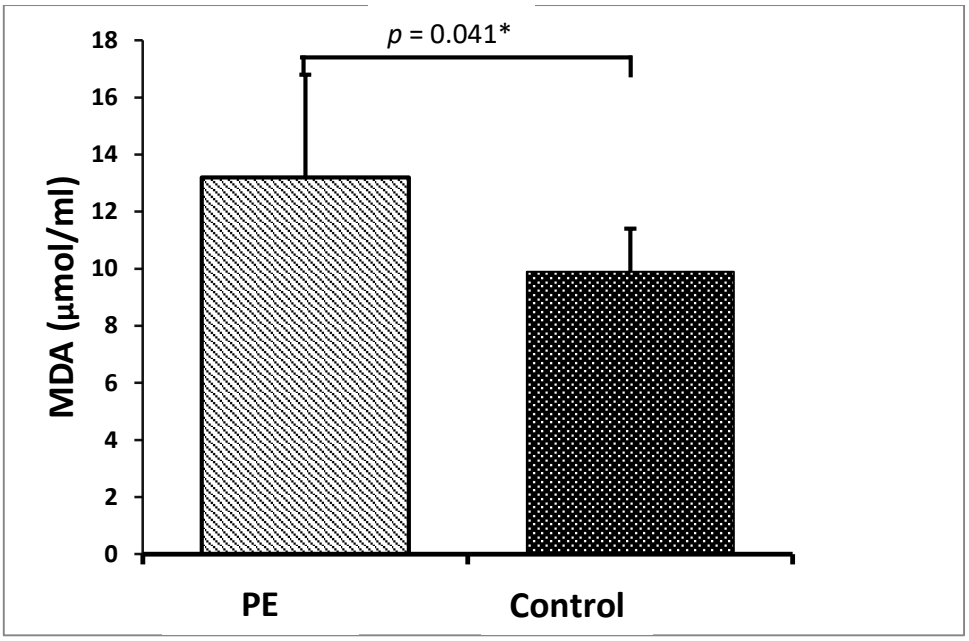

Fig 1: Activity of MDA in normotensive pregnant woman and preeclampstic pregnant women.

*p<0.05 is considered significant different

MDA = Malondialdehyde

PE = Preeclampsia

Paraoxonase-1 (PON-I) Activity

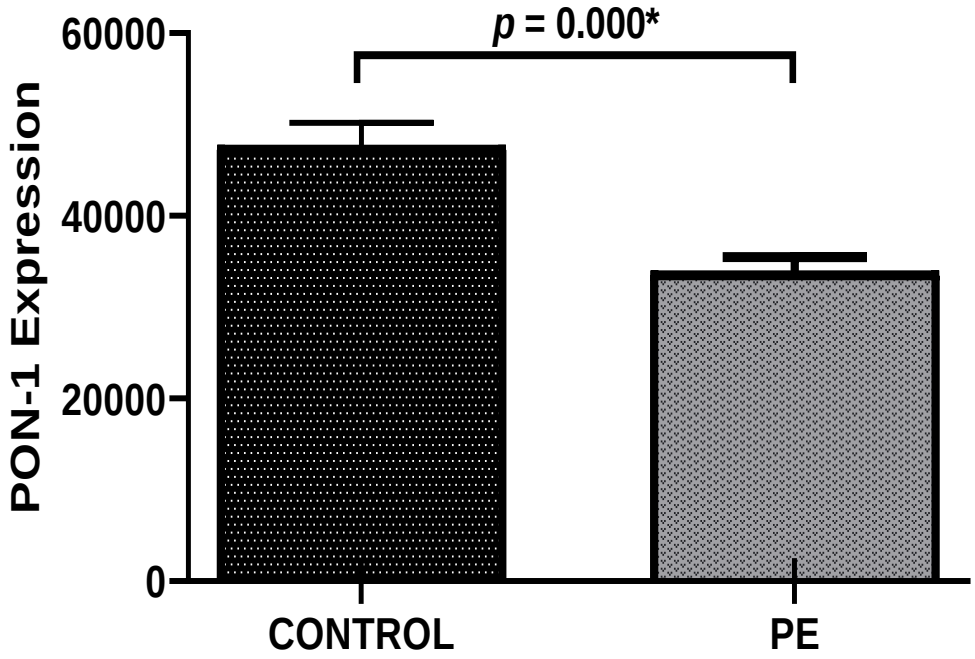

Fig 2: Expression of PON 1 in normotensive pregnant woman and preeclampstic pregnant women.

*p<0.05 is considered significant different

PON 1 = Paraoxonase-1

PE = Preeclampsia

Discussion

Pre-eclampsia (PE) is one of the main causes of maternal and fetal morbidity and mortality in the world, causing nearly 40% of births delivered before 35 weeks of gestation. The research therefore, evaluated lipid peroxidation and the expression of paraoxonase-1 (pon-1) gene in patients with preeclampsia

The result obtained from this study revealed a significant increase in Malondialdehyde (MDA) level in preeclamptic pregnant women when compared with normotensive pregnant women.

The significant increase in MDA level signifies the presence of lipid peroxidation and oxidative stress in these patients. This findings thus agrees with a previous study by Mohanty *et al*. (2006); the study showed that serum MDA levels were raised significantly in women with mild preeclampsia (P<0.01) and in women with severe preeclampsia (P<0.01) in

comparison to normal. The study suggests that in hypertensive disorders of pregnancy, there is an imbalance between lipid peroxidation and antioxidant status because of oxidative stress.

The study also revealed a significant decrease ($p<0.05$) in the expression of Paraoxonase-1 (PON-1) gene in preeclamptic pregnant women when compared with normotensive pregnant. PON-1 gene coded for paraoxonase-1, an HDL associated antioxidant enzyme whose activity is decreased in conditions associated with oxidative stress. This is in line with an earlier study by Hubel *et al.* (1996) which revealed that serum PON1 activity was decreased in women with severe pre-eclampsia. The decrease expression of PON-1 gene and consequently the decreased activity of paraoxonase could play a significant role in the development of PE. The findings of this study was also corroborated by Demir *et at*. (2011) where it was shown in their study that PON1 activity was significantly lower in pre-eclamptic subjects compared with controls; the study concluded that decreased PON1 activity may help to maintain levels of ApoB-containing lipoproteins within the normal range because of its decreased antioxidant activity.

The principal action of PON-1 is the protection of acute toxicity and oxidative stress involved in the development of atherosclerosis. PON-1 also has a role in the hydrolysis of organophosphate insecticides and nerve agents (Costa *et al*., 2011; Draganov *et al*., 2005). There is evidence that shows that preeclamptic women have lower levels of serum HDL, higher level of serum triglycerides, and lower level of serum Apo-A1 than control women (Kim *et al.* 2007), and this has been correlated with lower PON-1 serum activity in preeclamptic pregnant women (Uzun *et al*. 2005) as it was also shown in the present study.

Furthermore, PON-1, an HDL associated antioxidant enzyme proceeds as a protective factor against oxidative stress and LDL oxidation. Genc *et al*. (2011) in their study documented low PON-1 serum levels compared to the controls, which is line with the findings of the current study. In contrast, Yaghmaei *et al*. (2011) found high PON-1 activity in PE, whereas Sarandöl *et al*. (2004) study disclosed the insignificant difference in PON-1 activity in mild-to-severe PE compared to the normal pregnancy. It has been shown that PON-1 is linked with HDL and protects HDL from oxidation due to the ability of PON-1 to hydrolyze oxidized phospholipid (Al-Kuraishy *et al*. 2018).

CONCLUSION

The significant increase in the plasma level of Malondialdehyde in preeclamptic pregnant women indicates lipid peroxidation and oxidative stress in these patients. The decreased expression of PON-1 gene and consequently the its protein parooxonase-1 and HDL associated antioxidant could be responsible for the presence of oxidative stress and its associated complications in PE patients as revealed in this study. This is also a pointer to the fact that this gene could play significant role in the pathogenesis of PE and its complications.

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