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Expression of some selected genes with possible link to the pathogenesis and disorders of preeclampsia

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Abstract
Preeclampsia (PE) is a disease associated with pregnancy and a major cause of morbidity and mortality worldwide. In spite of researches in understanding the pathogenesis of PE, the underline mechanism is not yet clear. This study was carried out to evaluate the expression of selected genes that may be linked to pathways associated with the pathogenesis and disorders of PE namely leptin, Beta-hexosaminidase A and B, FMS related Tyrosine Kinase1 (FLT1), Cycline-dependent kinase inhibitor 1C(CDKN1C), Cytochrome P450 family II subfamily A member 1(CYPIIA1), Cytotoxic T-lymphocyte Associated Protein-4(CTLA-4). A total of 120 participants recruited from the Antenatal Clinic of Obsteric and Gyneacology department of University College Hospital Ibadan Oyo State Nigeria were used for this study. They were between the ages of 18 to 45 years and were grouped into 2 groups. Group 1 were 60 apparently healthy pregnant women in their 2nd trimester, Group 2 were 60 freshly diagnosed preeclamptic women in their 2nd trimester. Blood samples of the participants was taken and prepared to obtain total RNA using Quick-RNA MiniPrep™ Kit (Zymo Research). The RNA was converted to cDNA using ProtoSript First Strand cDNA Synthesis Kit(NEB). The result of the study shows that leptin, CDKN1C, CYPIIA and CTLA4 mRNA expression was significantly ($P<0.05$) high in PE patients than in non- PE pregnant women. There is however no significant difference $p<0.05$ in the mRNA expression of FLT1 gene in both test and comtrol. The expression of these genes and their roles in pathways linked to the etiology of PE could provide insight into the mechanism involved in the pathogenesis of PE and subsequently early diagnosis and reduction in prevalence.

Keywords: Preeclampsia, pregnancy, genes, pathways

Introduction
Pre-eclampsia (PE) is a medical complication and the most frequently occurring in pregnant women worldwide and one of the main causes of both fetal and maternal morbidity and mortality. PE affects around 10% of all pregnant women worldwide associated with risk of cardiovascular complications and death in later life [1, 2]. Most PE is characterised with abnormal placental implantation that defines the severity and the gestational age of presentation of the disease. The severity of PE is induced by the degree of inflammatory response taking place as a result of increase oxidative stress, generated by increased level of inflammatory and trophoblast factors in maternal circulation. This result in systemic endothelial dysfunction, hypertension, proteinuria and edema which are the major clinical manifestation of the disease [3]. The occurrence of PE reported within families is opening a possibility of genetic link to this disorder. It seems to represent a genetic disorder occurring as a result of possible defect to certain genes that are linked to the pathogenesis of PE. Genes that are plausible includes those associated with renin-angiotensin system (RAS), the generation or inactivation of reactive oxygen species, lipid peroxidation and immune factors which plays major roles in the pathogenesis of PE and its complications [4, 5].
Leptin gene provide instruction for making leptin, a hormone synthesized at many sites within the body with leptin receptors such as adipocytes and enterocytes that help to regulate energy balance and plays key roles in many physiological processes. In humans, maternal peripheral leptin levels was demonstrated to increase during pregnancy and its production hypothesised to be modulated by reproductive hormones [6]. In uncomplicated pregnancies, a two to four fold increase in leptin levels has been observed, peaking in second trimester and

declining post-partum. Low serum Leptin concentration has been observed in women suffering from miscarriage. The mechanism of gestational increase of leptin is yet to be explored, which may indicates that leptin might play crucial role in the outcome of pregnancy [7].

Beta-hexosaminidase A and B (HEXB) gene codes for a protein which is a sub unit of two related enzymes: beta-hexosaminidase A and B found in lysozyme and help to breakdown toxic substances, fatty compounds (sphingolipids) and complex sugars.

FMS related tyrosine kinase 1 (FLT-1) gene encodes a member of a vascular endothelial growth factor receptor (VEGFR), a receptor tyrosine kinase which bind to VEGFR-A, VEGFR-B and placental growth factor. In PE, higher amounts of soluble fms-like tyrosine kinase 1 (sFLT-1) are produced in the placenta. sFLT-1 competitively binds to placental growth factor (PIGF) and vascular endothelial growth factor (VEGF) creating an angiogenically imbalanced vascular environment that prevents proper endothelial preservation [8, 9].

Cytochrome P_{450} family II subfamily A member 1 (CYPIIA1) gene encodes a protein which is a member of the cytochrome P_{450} superfamily of mono-oxygenases which is located in the inner membrane of mitochondrial and catalyses the conversion of cholesterol to pregenolone, the first and rate limiting step in the synthesis of steroid hormones. This process could lead to low level of free intracellular cholesterol in individual with high expression of CYPIIA1 proteins and subsequently low incidence of cardiac events [10].

Cytotoxic T-lymphocyte associated protein 4 (CTLA-4) gene codes for CTLA-4 protein, a receptor that functions as an immune response down regulator. They are expressed in conventional T-cells after activation especially in cancer cells, causing their silencing or death [11]. Therefore, CTLA-4 acts as a key down regulator of the immune response and represents an excellent candidate for an autoimmunity gene. Earlier report has linked polymorphism in the CTLA-4 gene with the development of placental abruption and preeclampsia [12].

Methodology

Participants

A total of 120 participants were recruited from the Antenatal clinic of Obstetrics and Gynecology department of University College Hospital, Ibadan, Oyo State, Nigeria and Ekiti State teaching Hospital Ado Ekiti, Nigeria. They were arranged into two groups with group 1 made up of sixty apparently healthy pregnant women in their 2nd trimester, while group 2 contains 60 freshly diagnosed pre-eclamptic patients at second trimester serve as test.

Study Area and Ethical Considerations

The University College Hospital (UCH) Ibadan, Oyo State, Nigeria was used for this study and Ethical approval was obtained from Ethics and Research Committee of UCH, Ibadan where blood samples for the research were collected.

Sample Collection

Blood sample (15 ml) was collected from each participant into plain vacutainers with RNA and DNA shield in order to preserve the blood. The samples were then stored at -20 ºC prior to analysis.

Biochemical Analysis

Extraction of RNA

The Total RNA was extracted from the plasma of the stored studied groups sample using ZYMO-RNA extraction kit. The concentration of total RNA was afterwards determined by UV/VIS spectrophotometer made by A&E Lab.

Gene Expression Analysis

The expression of all the genes considered in this study in the 2 groups, was analyzed using reverse transcription-PCR (RT-PCR) assay. The assay was performed using an optimization Template (cDNA) Reactions (50 µL final volume) contained 5 µL of cDNA, 2 µL each of sense and antisense primers, 200 Mm of each deoxynucleotide, 5 µL of 10×Taq polymerase buffer, and 1.25 U Taq polymerase. The cycling conditions were were: 94 °C pre-denaturation for 5 min, 94 °C for 30 s, annealing 55 °C for 30 s and Extension 72 °C for 30 s and then 5 min at 72 °C by 30 cycles using Eppendorf Master cycler Hamburg. PCR products were separated by electrophoresis in 2% agarose gels and visualized under blue light transilluminator. The product intensities were quantified by computer using image mRNA expression levels were normalized to that of the housekeeping gene, β-actin.

Table 1: Showing the forward and reverse primers of the selected genes

Gene Forward primer Reverse primer
FLT1 5'-CCGGCTCTCTCTATGAAAGTGAAG-3' 3'-CGAGTAGCCACGAGTCAAATAG-5'
CDKN1C 5'-CCAAAGGCACTCTCCATCTC-3' 3'-TATGGCAGCTACAGCTTGTG-5'
CYP11A1 5'-CCAGCATCAAGGAGACACTAAG-3' 3'-CAGTGTCTTGGCAGGAATCA-5'
CTLA-4 5'-GGAGGAGCTCAGGACACTAATA-3' 3'-CAGCACAATTCCACGCAATC-5'
LEPTIN 5'-CCTCTGATACCCAGAGCATTAC-3' 3'-CCTCACCTCCTTCAAACTCTAC-5'
HEXB 5'-GGCGGTACTCAGAAACAGAAA-3' 3'-GAGGCCATAATCTTGGAGTGAG-5'

Statistical Analysis

The distribution of the analyzed expression data gotten from gel images quantified by densitometry method using Image J launcher (version 1.4.3.7) were subjected to statistical analysis using Graph pad prism version 7. The results obtained were also grouped and expressed as mean ± Standard Deviation (SD). Two-way analysis of variance (ANOVA) was used to compare variable across the test and control groups. Student t-test was used to compare variables between the test and control groups, p value < 0.05 was considered significant.

Results

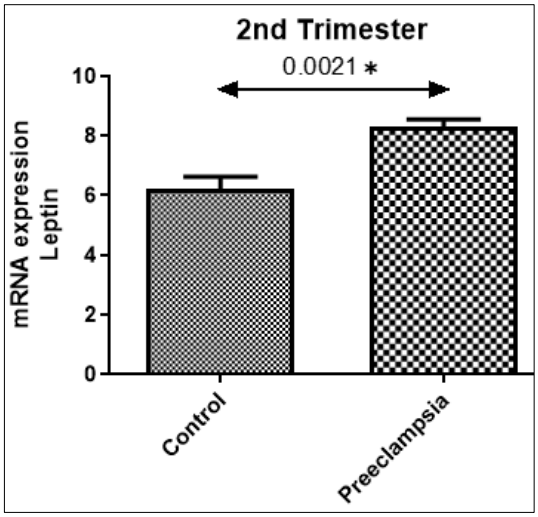

Fig 1: Comparism of the mRNA expression of leptin between normotensive pregnant women and women with Preeclampsia in their second trimester.
* Indicate significant difference at p<0.05

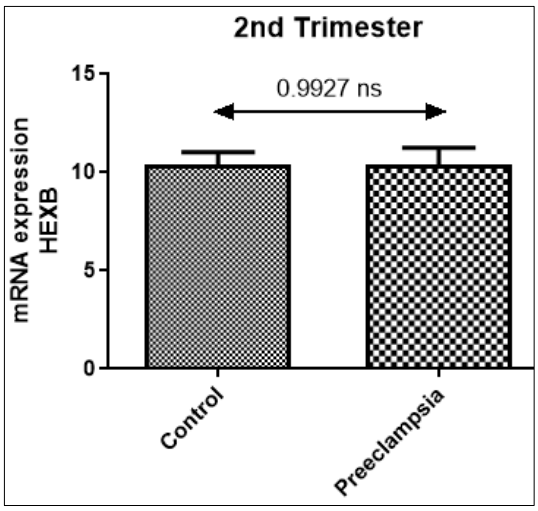

Fig 2: Comparism of the mRNA expression of HEXB between normotensive pregnant women and women with Preeclampsia in their second trimester.

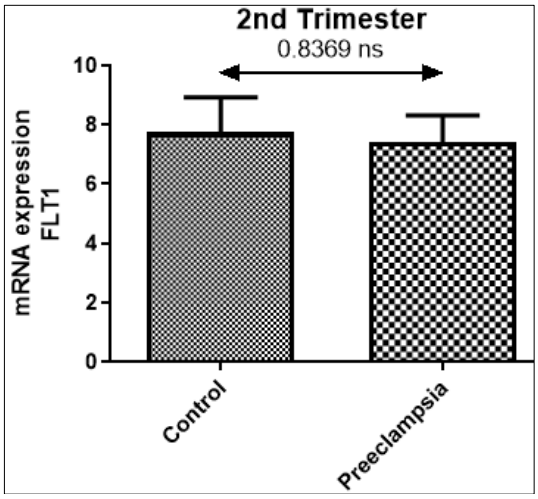

Fig 3: Comparism of the mRNA expression of FLT1 between normotensive pregnant women and women with Preeclampsia in their second trimester.

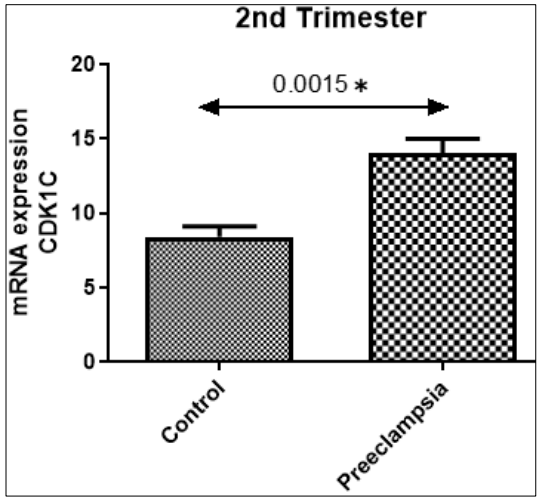

Fig 4: Comparism of the mRNA expression of CDK1C between normotensive pregnant women and women with Preeclampsia in their second trimester.
* Indicate significant difference at *p*<0.05

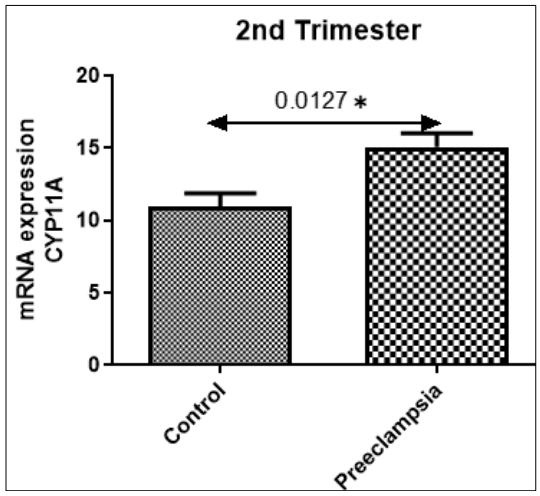

Fig 5: Comparism of the mRNA expression of CYP11A between normotensive pregnant women and women with Preeclampsia in their second trimester.
* Indicate significant difference at p<0.05

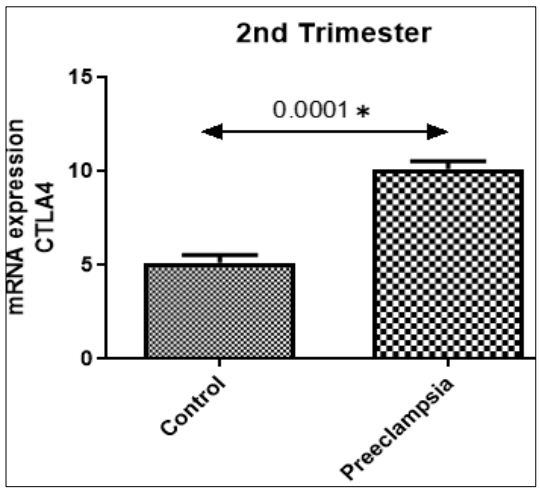

Fig 6: Comparism of the mRNA expression of CTLA4 between normotensive pregnant women and women with Preeclampsia in their second trimester.
* lndicate significant difference at *p*<0.05

Discussion

Preeclampsia (PE) remains a major cause of maternal and neonatal morbidity and mortality worldwide. The familial component which is very clear is suggesting that the condition may be partly attributable to genetic susceptibility. The result of this study reveals a significant (p<0.05) mRNA expression of Leptin, CDK1C, CYPIIA1, CTLA4 genes while there was no significant difference in that of HEXB and FLT1 genes in PE patients when compared with the control subjects.

The significant expression of leptin gene in these patients correlates with high production of leptin protein which usually characterize pregnancy and is said to be hormone dependent and placental induced [13]. High leptin level has been found to contribute to hyperrenineamia and hypoaldosterionism which lead to high level of angiotensin and subsequently high blood pressure a major disorder of PE [14]. The undisputed role the placental is playing in the development of PE may be pointing in the direction of leptin for a possible role in the pathogenesis and complications associated with PE which still need to be elucidated. The mechanism of gestational increase in leptin level is also not yet explored.

The CDKNC gene which was also significantly expressed in these patients is known to code for the protein $p57^{Kip2}$. In both humans and mice, $p57^{Kip2}$ is a powerful inhibitor of numerous cyclin/cyclin dependent kinase complexes (CDK), a paternally imprinted gene. Cyclins and cyclin dependent kinases control the cell cycle of trophoblastic cells and other cells (CDKs). The significant expression of this gene in these patients could be due to the abundant presence of its transcript in the placenta and could also be critical in the pathogenesis of the disease. Abnormal placentation was reported in mouse with CDK1C mutation [15], however, the action of CDKN1C in human placentation may differ because the QT-box in the transcript in human has proline-alanine (PAPA) repeats unlike the proline-rich and acidic domain found in mouse [16]. The exact role of CDKN1C in human placentation still need to be further elucidated.

The CYP11A1 gene which was also observed to be significantly expressed in these patients coded for CYP11A1 protein which is one of the various cytochrome P_{450} enzymes that is involved in the biosynthesis of steroid hormones from cholesterol and therefore a useful steroid metabolic biomarker. CYPA11A1 is the most important and rate limiting enzyme for the conversion of cholesterol to pregnenolone the rate limiting step in the biosynthesis of steroid hormones. High level of steroid hormones are associated with hypertension and endothelial dysfunction which are features associated with PE [17]. Similar outcome was also reported by [18]. This gene and its protein could present a new platform for the synthesis of drugs for better management of this disease and its complications.

The cytotoxic T-lymphocyte- associated protein 4 (CTL-4) gene expressed in these patients coded for a protein which found expression on T-cells only after antigen stimulation. This protein acts as a key down regulator of the immune response by interacting with activated T-cells carrying a CTL-4 receptor causing their death or silencing and plays key roles in maintaining maternal/fetal tolerance [19]. The maternal immune system and the placenta help the fetus to avoid immune attack in a normal pregnancy, therefore, the altered immune response due to the high expression of CTL4 protein in these patients could be key in the pathogenesis of PE and its complications which could offer a new line of research in the prevention and management of the disease.

HEXB and FLT1 gene mRNA expression was not significantly different in both patients and control subjects examined in this study as shown in figure 4.2 and 4.3, HEXB gene coded for hexosaminidase B a protein that plays significant role in the degradation of ganglioside whose accumulation in neurons has been implicated in neurodegenerative disorders but has not been linked to PE. FLT1 gene on the other hand coded for a member of vascular endothelial growth factor receptor (VEGFR) family which binds to VEGFR-A, VEGFR-B and placenta growth factor and play key roles in angiogenesis, vasculogenesis and placenta development.

Conclusion

The significant expression of Leptin, CDK1C, CYPIIA1 and CTLA4 genes considered in this study in PE patients could mean a possible link to the etiology, pathogenesis and proper management of the disease. However further studies may be required to ascertain the veracity and the pathophysiological link to the development of the disease and possibly provide useful information on its prevention and management.

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