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Original Research Article

Lack of nitric oxide bioavailability in early pregnancy predisposes to dyslipidemia and surges preeclampsia and fetal growth retardation

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ABSTRACT

Background: Hyperlipidemia has been reported in preeclampsia (PrE) and is linked to poor pregnancy outcome and long-term cardiovascular complications. This study aimed to elucidate the relationship between nitric oxide (NO) and blood lipids levels during normal pregnancy and in N^G-nitro-l-arginine methyl ester (L-NAME) - induced preeclampsia before and after magnesium sulphate ($MgSO_4$) therapy and its effect on the pregnancy outcome.
Methods: Forty female Wistar rats were divided into four groups: non pregnant (NP) group - non pregnant healthy rats receiving no treatment, control pregnant (Con-P) group - control pregnant rats receiving no treatment, pregnant (PE) group - pregnant animals with untreated PrE, and the pregnant $MgSO_4$ (PE-Mg) group - pregnant animals with PrE-treated with $MgSO_4$. The nitric oxide synthase inhibitor L-NAME was used to induce experimental model of PrE in the PE and the PE-Mg groups. The changes in total NO production, total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL-C), high density lipoproteins (HDL), LDL-C/HDL-C ratio, soluble vascular endothelial growth factor receptor-1 (sVEGFR1) also known as sFlt-1, blood pressure, kidney functions, body weight, and pregnancy outcome were assessed.
Results: Decreased NO production in the PE group was associated with elevated TC, TG, LDL-C/HDL-C ratio, hypertension, proteinuria, increased urea, creatinine, and sFlt-1 levels, and poor pregnancy outcome demonstrated by high pup mortality rate and low birth weight. Increased NO production in the PE-Mg group treated with $MgSO_4$ therapy was associated with decreased signs of preeclampsia and hypolipidemia and increased pup viability and birth weight.
Conclusions: NO bioavailability is crucial for the homeostasis of the lipid profile in normal pregnancy and the prevention of preeclampsia. Routine periodic assessments of the blood lipid profile and the NO production in the pregnant females may be a helpful tool in early prediction of preeclampsia.

Keywords: Preeclampsia, Nitric oxide, Hyperlipidemia, Low birth weight, Endothelial dysfunction, Magnesium sulphate

INTRODUCTION

Preeclampsia (PrE) is a pregnancy specific syndrome that affects around 3-6% of mothers globally and up to 10% in developing countries.[1] The burden of PrE is linked to its role as the principal cause of fetal prematurity, maternal morbidity, and almost 15% of the pregnancy- associated deaths.[1,2]

The pathogenesis of PrE is thought to act at three levels: defective placentation, placental ischemia, and endothelial dysfunction.[3] The ischemic placenta is hypothesized to be associated with enhanced formation of vasoconstrictor substances and lipid peroxidation products and decreased bioavailability of the vasodilator molecules such as nitric oxide (NO) and prostacyclin.[4] Evidence of endothelial dysfunction as an early event in the process of PrE

suggests that it is a possible cause and not a result of the disorder.[5] This supports the hypothesis stated by Roberts and colleagues more than a decade ago that widespread dysfunction of the maternal vascular endothelium is a key factor associated with PrE.[6] Endothelial abnormalities, in turn, cause glomerular endotheliosis, impaired renal pressure natriuresis, and increased total peripheral resistance resulting in hypertension and proteinuria.[7]

Despite the clinical manifestations of generalized systemic involvements in patients of PrE; the disease seems to be self-limited condition that resolves after delivery of the placenta. However, this is not the end of the story: long term maternal complications like hypertension, renal diseases and proteinuria are more frequent in affected patients.[9,10] This may be due to persistent subtle endothelial damage or as a consequence of the vascular risk factors.[11] Although the etiology of PrE is still a matter of debate among scientists, there is rising research interest in the role of disordered plasma lipids in the endothelial dysfunction associated with the complicated pregnancy especially in the case of PrE.[12,13]

The physiological hormonal and metabolic changes of pregnancy favor enriching the blood with organic molecules including glucose, amino acids and fatty acids necessary for the growth and nutrition of the developing fetus.[14] The enhanced lipid turnover and metabolism is not unexpected to influence the circulating lipoproteins during this physiological process.[15] Moreover, loss of balance between the atherogenic and anti-atherogenic blood lipids in pregnant females will be associated with corresponding changes in the endothelial structure and function. The latter has its impact on the newly formed blood vessel in the developing placenta and consequently on the blood flow to the fetus.[15] In this regard, recent reports indicated that disturbed blood lipids in early pregnancy predisposes to pregnancy induced hypertension (PIH) and PrE and is hypothesized to be an indicator for long-term cardiovascular complications in the affected females.[16,17] Additionally, elevated triglyceride (TG) levels in the second and third trimesters of pregnancy was reported to be associated with gestational diabetes mellitus and preterm labor.[18]

Although multiple maternal clinical, historical, ultrasonic, and laboratory biomarkers are being utilized in the screening for PrE, the blood lipid profile is not included in the risk assessment of PrE such as the foetal medicine foundation algorithm.[19] Likewise, limited resources are available regarding the relationship between blood lipids and the vasodilator NO, which has a role in regulating the function of the endothelium and new blood vessels formation. Additionally, the effects of the changes in blood lipids on the pregnancy outcome regarding the fetal birth weight and viability need to be well investigated. In a previous publication of our laboratory, we reported a protective role of magnesium sulphate ($MgSO_4$) therapy on N^G-nitro L-arginine methyl ester (L-NAME)- induced PrE in rats demonstrated by decreased clinical and laboratory signs of PrE and increased NO production.[20] In continuation to our previous research on the etiological factors of PrE and the mechanisms of action of $MgSO_4$ therapy on this major health problem, this study aimed to elucidate the changes in the blood lipids in normal pregnancy and in L-NAME-induced PrE in rats and to clarify the relationship between NO bioavailability and the changes in the blood lipids, the clinical signs of PrE, the kidney functions and the pregnancy outcome. Additionally, we scrutinized the effects of $MgSO_4$, being one of the most effective therapies used in the treatment of severe PrE and prevention of eclampsia on the studied parameters.

METHODS

Animal experimental design

The research ethics committee of the College of Medicine King Saud University (KSU) approved the study protocol and the experimental design. All animal handling and procedures were in accordance with animal research: reporting of in vivo experiments (ARRIVE) guidelines and the recommendations of the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) and the regulations of the experimental animals care unit of the College of Medicine, KSU. The study was conducted between November 2020 and June 2021 in the research laboratory of the physiology department and the experimental animal care centre of the College of Medicine, King Saud University Medical City (KSUMC), KSU. The study included 40 female Wistar rats (weight 250-300 g) kept under standard laboratory conditions at 23^0 C temperature, 50±10% humidity, 12 hour light/dark cycles, and free access to rodent food and drinking water, ad libitum. The study involved 10 non pregnant (NP), and 30 pregnant animals divided equally into the following groups. Con-P group - control pregnant rats receiving no treatment, PE group - pregnant animals with L-NAME-induced preeclampsia, and PE-Mg group - pregnant animals with preeclampsia treated with $MgSO_4$ therapy.

The induction of normal pregnancy was carried out by housing the virgin female rats with male rats (1:1) overnight and confirmation of pregnancy was performed as detailed elsewhere.[20] In order to generate a preeclampsia-like condition, the animals in the PE and PE-Mg groups received 60 mg/kg/day L-NAME powder (Sigma chemical, St. Louis, MO) dissolved in 0.5 ml distilled water by oral gavage starting at day 13 of pregnancy through full term. At the same time, the animals in the NP and Con-P groups received 0.5 ml distilled water by oral gavage.[20] The (PE-Mg) group received $MgSO_4$ 500 mg/kg/day, subcutaneously (magnesium sulfate heptahydrate 500 mg/ml injections, Inresa Arzneimittel GmbH) from day 17 of pregnancy to full term (day 22). The animals in the NP and Con-P groups received 1.0 ml of 0.9% normal saline/day subcutaneously.[20] All treatments were administered at 8:00-9:00 am daily.

Assessments of systolic blood pressure and urine collection

Systolic blood pressure (BP) was recorded on the morning of day 20 of gestation by non-invasive blood pressure (NIBP) system using the tail–cuff plethysmography (Letica LE 5100, Panlab, Barcelone, Spain) from Panlab Technology for research–Spain). The average of three measurements was used as a single value for each rat at each time point. For urine collection the animals were kept individually in metabolic cages with no food intake but with free access to water from 2:00 pm of day 20 of pregnancy to 8:00 am next morning. This allowed for 18 hour urine collection. The collected urine was centrifuged at 3000 rpm for 10 min and the supernatant was used to measure the protein and the stable NO metabolites nitrite and nitrate (NO^{2-}/NO^{3-}) levels.

Pup delivery and blood sampling

At day 22 of pregnancy, the animals were weighted and anesthetized with ketamine (40 mg/kg body wt., IM) and xylazine (5 mg/kg body wt., IM). A midline laparotomy incision was performed to expose the uterine horns and the pups were removed. The number, weight, and viability of the pups were recorded. Blood samples were collected by cardiac puncture into plain test tubes and the serum was separated and kept at -20°C for further biochemical assays.

The biochemical assays

The following biomarkers were assayed in all the studied groups.

Blood lipid profile

Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were assayed calorimetrically by local commercial kits supplied by United Diagnostics Industry, Riyadh, KSA, according to the manufacturer's instructions. The levels of low-density lipoprotein-cholesterol (LDL–C) were estimated by the following formula given.[21]

$$LDL - C = [TC] - [HDL - C] - [TG/5]$$

Kidney function tests

Serum urea and creatinine levels were measured by specific calorimetric kits purchased from United Diagnostics Industry, Riyadh, KSA as per the instructions of the manufacturer. Urinary protein levels were detected with commercial dipstick strips and quantified by protein assay kit (Spinreact-Spain).

The protein in the samples reacts in acid solution with pirogallol red and molybdate to form a colored complex. The intensity of the color formed is proportional to protein concentration in the sample.[22]

Nitric oxide (NO) and sFlt1 levels

Special enzyme-linked immunosorbent assay (ELISA) kits produced by (R&D laboratories, USA) were utilized to measure NO and the soluble vascular endothelial growth factor 1 (sFlt1) levels according to the manufacturer instructions.

Total NO production was evaluated by measuring the stable end products of NO metabolism (nitrite and nitrate) in serum and urine utilizing the nitrate reductase enzyme and the Griess reagents and using ELISA reader according to the manufacturer instructions and as reported previously.[23]

Statistical analysis

The data was checked for normal distribution and screened for outliers and analyzed by the computer software GraphPad Prism.9.0 (GraphPad software, LLC). The variables were presented as mean±standard deviation (SD) and comparison between several groups was done using one-way analysis of variance (ANOVA) followed by the post Hoc Tukey's test. The relationship between different variables was assessed by Pearson's correlation analysis. Results were considered significant at $p<0.05$.

RESULTS

The blood lipid profile in the control pregnant and the preeclampsia animals

TC

As Figure 1a shows, the Con-P animals developed higher serum TC levels in comparison to the NP groups ($p=0.01$). However, the animals in the PE group exhibited greater increase in the TC levels in comparison to the NP ($p<0.0001$) and the Con-P groups ($p<0.0001$). Treatment by $MgSO_4$ was associated with significant reduction of the TC levels in the PE-Mg group in comparison to the PE group ($p<0.0001$). The TC levels were comparable ($p<0.05$) in the PE-Mg and the Con-P groups.

TG

The Con-P animals showed higher values of serum TG (134.9±29.04 mg/dl) in comparison to the NP group (74.13±25.92 mg/dl) but this did not reach a significant level ($p>0.05$**)** (Figure 1b).

The induction of preeclampsia was associated with significant increase in the serum TG in the PE group (310.4±86.42 mg/dl) in comparison to the NP group and the Con-P group ($p<0.0001$). Treatment by $MgSO_4$ was associated with significant reduction ($p<0.0001$) of TG levels in the PE-Mg group (172.82±61.72 mg/dl) in comparison to the PE-group showing no significant difference from the Con-p group ($p>0.05$).

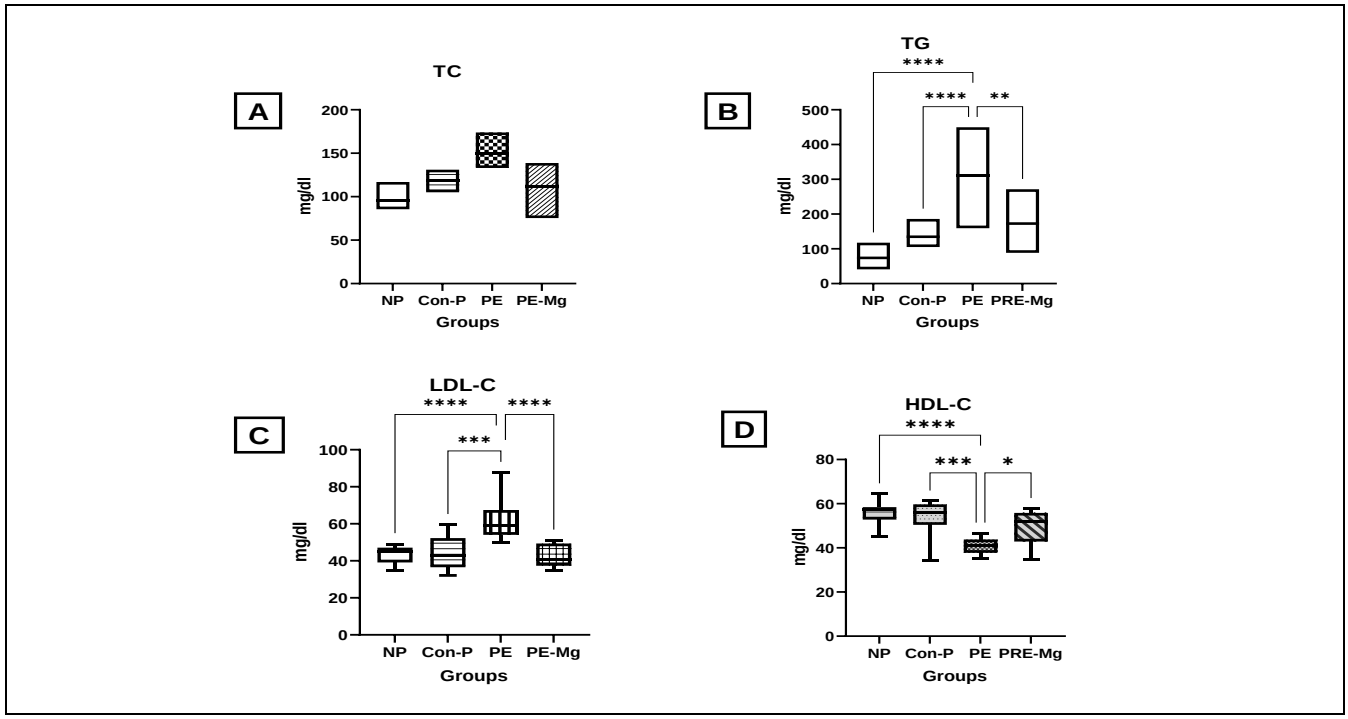

Figure 1: (a) The changes in serum total cholesterol (TC), (b) triglycerides (TG), (c) low density lipoprotein cholesterol (LDL-C), and (d) high-density lipoprotein cholesterol (HDL-C) in the control non pregnant (NP), control pregnant (Con-P), preeclampsia (PE) and preeclampsia-treated with magnesium sulphate (PE-Mg) groups at the end of the study.

Data is presented as mean±SD, ****p<0.00001, ***p<0.0001, **p<0.001, *p<0.05

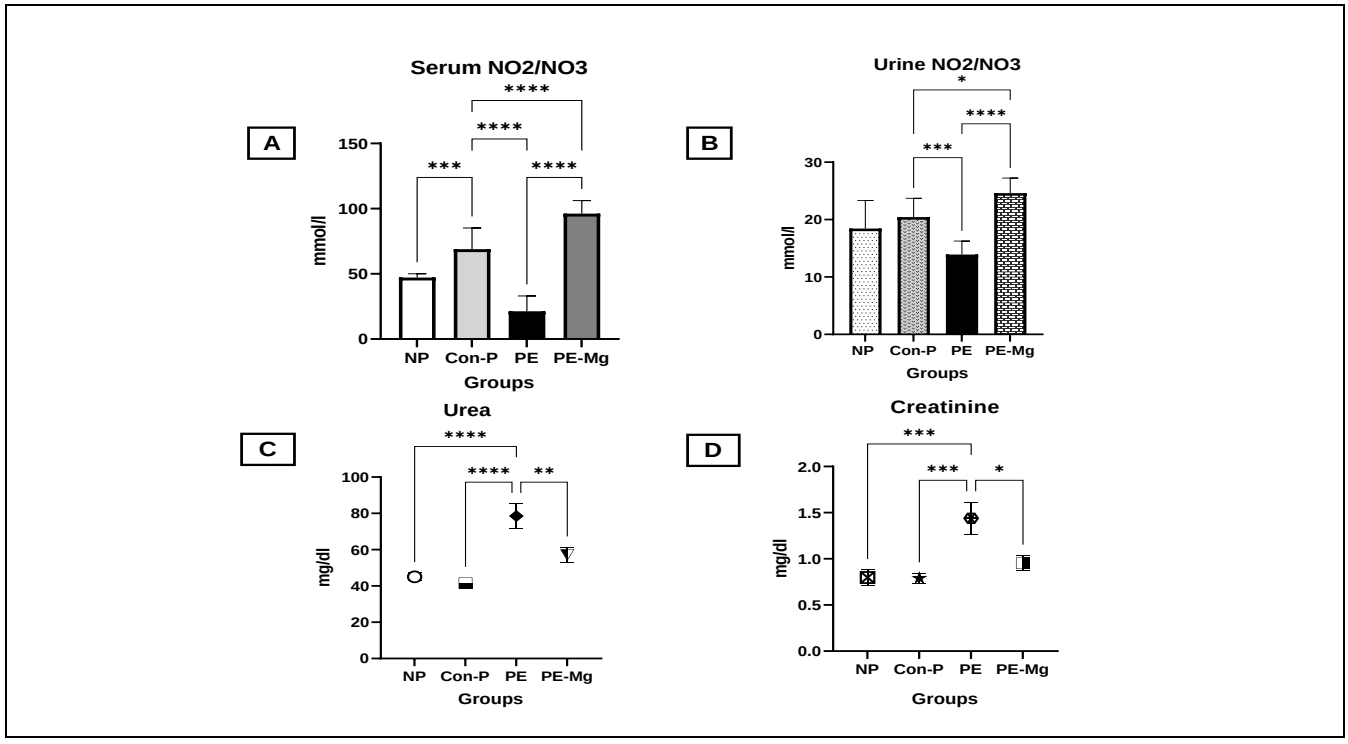

Figure 2: The changes in total nitric acid (NO) metabolic end products nitrite and nitrate (NO2-/NO3-) in the (a) serum, (b) urine, (c) serum urea and (d) serum creatinine levels in the control non pregnant (NP), control pregnant (Con-P), preeclampsia (PE), and preeclampsia-treated with magnesium sulphate (PE-Mg) groups at the end of the study.

Data is presented as mean±SD, ****p<0.00001, ***p<0.0001, **p<0.001, *p<0.05

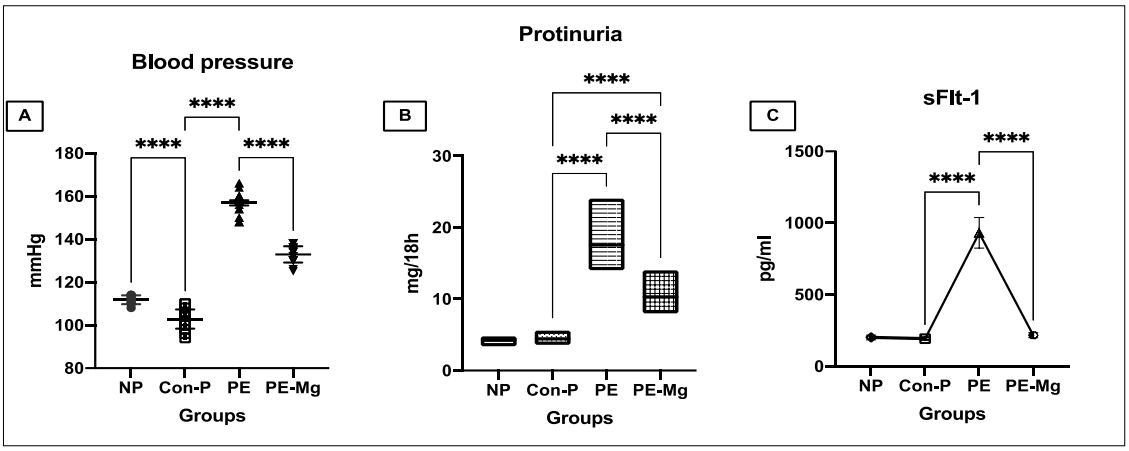

Figure 3: The changes in (a) blood pressure, (b) proteinuria, and (c) the serum levels of soluble vascular endothelial growth factor receptor-1 (sFlT-1) levels in the control non pregnant (NP), control pregnant (Con-P), preeclampsia (PE), and preeclampsia-treated with magnesium sulphate (PE-Mg) groups at the end of the study.

Data is mean±SD, ****p<0.00001

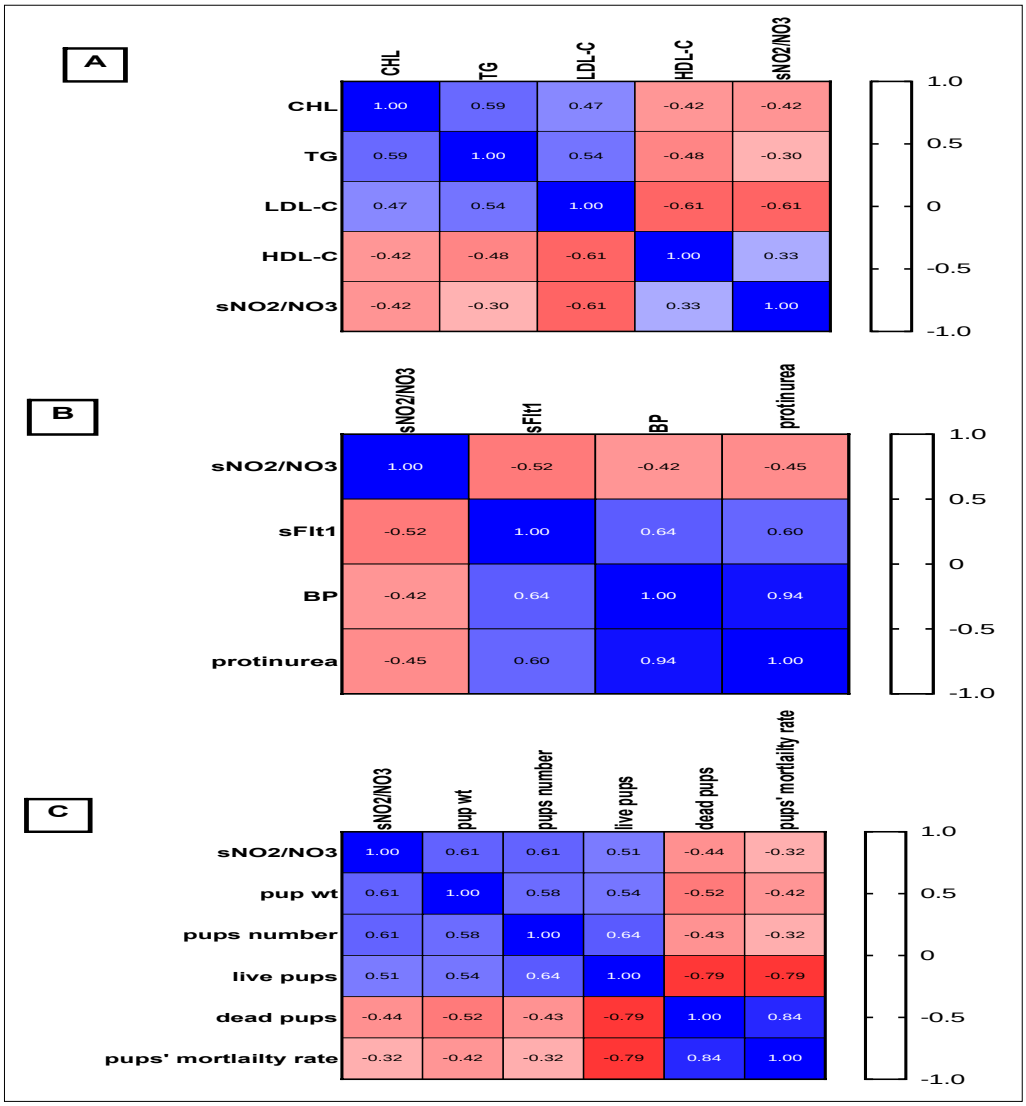

Figure 4: The Pearson's correlation analysis study of the relation between NO^{2-}/NO^{3-} and (a) blood lipids, (b) markers of preeclampsia, and (c) pregnancy outcome.

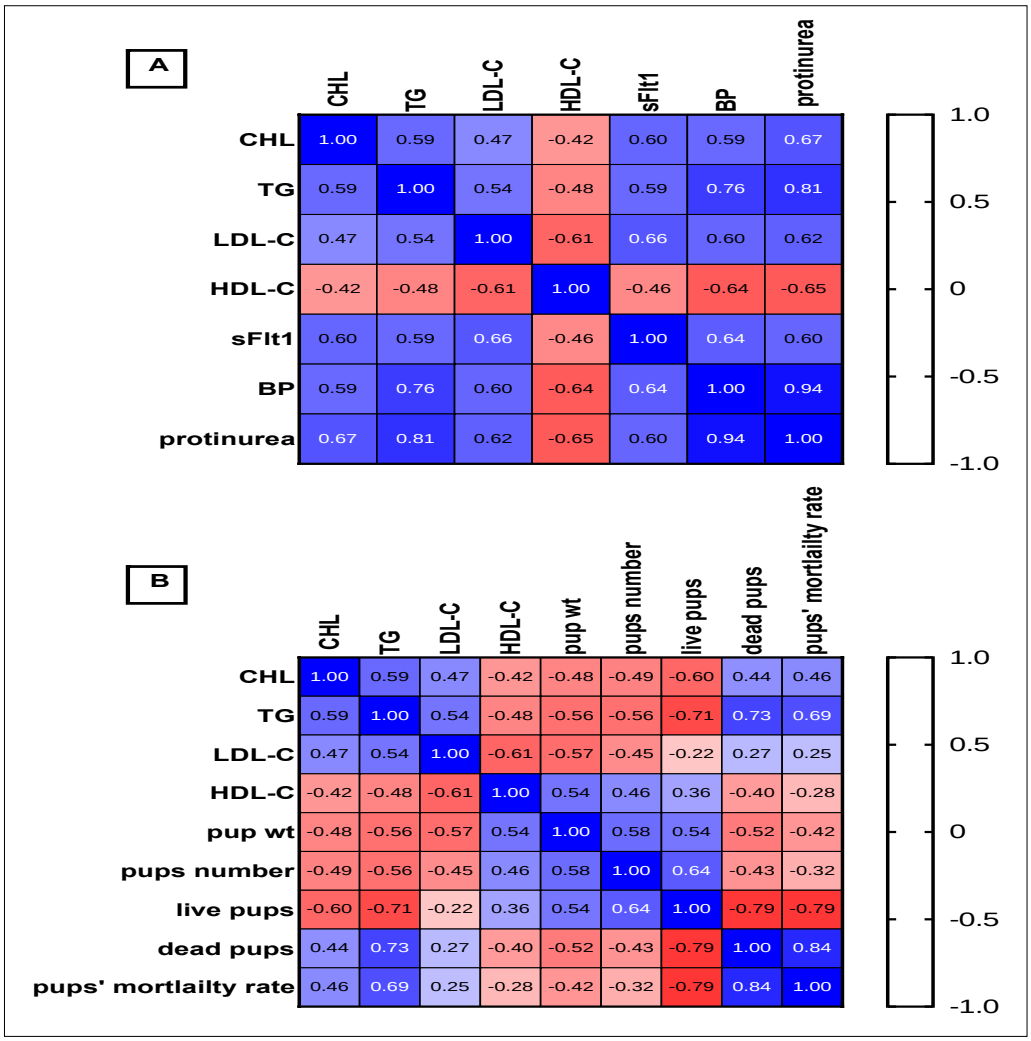

Figure 5: The Pearson's correlation analysis study of the relationship between the blood lipids and (a) markers of preeclampsia, and (b) pregnancy outcome.

LDL-C

Figure 1c shows that the LDL-C levels exhibited no significant change in the Con-P animals in comparison to the NP group ($p<0.05$). The PE group showed increased LDL-C levels in comparison to the NP ($p<0.0001$) and the Con-P group ($p=0.0001$). However, the $MgSO_4$- treated PE-Mg group showed significant reduction of the LDL-C levels ($p<0.0001$) in comparison to the PE group.

HDL-C

The HDL-C presented in Figure 1d displayed no significant change in the normal pregnant (Con-P) group in comparison to the NP group ($p>0.05$). However, the PE group showed significant reduction in the HDL-C in comparison to the NP group ($p<0.0001$) and the Con-P group ($p=0.0005$). The PE-Mg group showed HDL-C levels that were comparable to the NP and Con-P groups ($p>0.05$).

LDL-C/HDL-C ratio

The Con-P group showed no significant change in the LDL-C/HDL-C ratio (0.85±0.29) in comparison to the NP group (0.78±0.14) ($p>0.05$). However, the PE group showed significantly higher LDL-C/LDH-C ratio (1.53±0.36) in comparison to the Con-P group ($p<0.0001$). The PE-Mg showed significant decline in the LDL-C/HDL-C ratio (0.87±0.24) in comparison to the PE group ($p<0.0001$).

Changes in NO production and kidney functions

The total NO production determined by the stable end products metabolites nitrite (NO^{2-}) and nitrate (NO^{3-}) levels were significantly higher ($p=0.007$) in the serum and urine of the Con-P animals in comparison to the NP group Figure 2a and b with no significant change in serum urea and creatinine levels Figure 2c and d ($p>0.05$ for all). However, the PE group showed impaired total NO^{2-}/NO^{3-} levels in serum ($p<0.0001$) and urine ($p=0.001$) and

increased serum urea (p<0 0.0001), creatinine (p=0.0009, 0.0008) in comparison the Con-P and NP groups. The $MgSO^{4-}$ treated PrE-Mg showed marked increase in the serum and urine NO^{2-}/NO^{3-} levels (p<0.0001 for both) (Figure 2a and b) combined with significant decrease in serum urea (p=0.008) and creatinine (p=0.01) compared to the PE group.

Signs of preeclampsia

The Con-P animals exhibited significant reduction in the BP at day 20 of pregnancy in comparison to the NP group (p<0.0001) (Figure 3a), with no significant change in the urinary protein loss (Figure 3b) or sFlt1 levels (p>0.05) (Figure 3c). On the contrary, the PE group showed marked hypertension and proteinuria and increased sFlt-1in comparison to the NP and the CON-P groups (p<0.0001 for all). Treatment with $MgSO_4$ resulted in significant reduction of the BP, proteinuria and sFlt1 levels in the PE-Mg in comparison to the PE group but it remained significantly higher than the NP and the Con-P group (p<0.0001 for all).

Morphometric criteria of the pregnant animals and their pups

The animals in the PE group had (18.28±6.50) percentage change in body weight (% BW) calculated as final body weight minus initial body weight divided by initial body weight, in comparison to the NP (22.95±2.64) and the Con-P groups (24.13±4.46) (p>0.05 for both). However, the % BW in the PE-Mg group (24.73±5.93) was greater (p=0.03) than the PE group and comparable (p>0.05) to the Con-P group. Furthermore, the PE group exhibited significantly low birth weight of the pups (1.82±0.74 g), and higher pup mortality rate (95% CI: 24.88 to 91.51) (in comparison to the Con-P group (6.50±1.59 g) and (95% CI: 0.00 to 0.00) (p<0.0001 and p=0.0003 respectively). Treatment by $MgSO_4$ in the PE-Mg group was associated with increased pup's birth weight (5.03±1.49 g) in comparison to the PE group (p<0.0001). The mortality rate in the $MgSO_4$ treated group (95% CI: 0.89 to 26.77) was significantly lower in comparison to the PE group (p=0.005).

Correlation analysis

The relationship of $sNO2^-/NO3^-$ to blood lipids, markers of preeclampsia, and pregnancy outcome

Serum NO^{2-}/NO^{3-} showed inverse correlation with TC (r=-0.42, p=0.007), TG (r=0.30, p=0.05), LDL-C (r=-0.61, p=0.005), sFlt-1 (r=-0.52, p=0.0005,) and BP (r=-0.41, p=0.007), proteinuria (r=-0.45, p=0.003) and the number of dead pups (r=-0.43, p=0.01). However, it correlated directly with HDL-C levels (r=0.32, p=0.03), the number of pups (r=0.61, p=0.0003), the pup birth weight (r=0.61, p=0.003), number of live pups (r=0.51, p=0.003) (Figure 4 a-c).

The relationship of the blood lipids to the signs of preeclampsia and the pregnancy outcome

The levels of TC, TG and LDL-C correlated directly with the BP, proteinuria and sFlt-1 levels in the studied groups. However, the HDL-C levels correlated negatively with the signs of preeclampsia (Figure 5a). Similarly, the serum TC, TG and LDL-C levels correlated inversely with number of live pups, pup's birth weight and positively with the number of dead pups and mortality rate. Alternatively, the serum HDL-C correlated negatively with the pup mortality rate and positively with the number of pups and the pup's birth weight (Figure 5b).

DISCUSSION

NO is a major endothelial relaxing factor that has a crucial role in the normal pregnancy through its vasodilator, antiplatelet, and hypolipidemic actions.[24] NO contributes to the regulation of placental blood flow through stimulating the cytotrophoblast endovascular invasion and placental development.[25] It also activates the mitogenic effect of vascular endothelial growth factor (VEGF) in the angiogenic and vasculogenic process during pregnancy.[25] The control pregnant animals in the current study exhibited increased NO production in comparison to the NP animals. This coincides with similar results in pregnant females that were reportedly attributed to increased expression of renal constitutive (nNOS) and increased NO production from arginine via the endothelial endothelin B receptors augmented by orexin hormone released from the ovaries under the influence of human chorionic gonadotrophin.[26,27] The hypotensive pattern of the blood pressure demonstrated in the Con-P group of animals reflected the systemic vasodilation induced by the increased serum NO levels and the hormones of pregnancy including estrogen and relaxin.[27]

The role of NO in the pathogenesis of PrE and its level in these cases is still a matter of controversy between investigators.[26,28,29] Blocking the eNOS by L-NAME in the PE group of animals resulted in significant inhibition of NO production indicated by decreased NO^{2-}/NO^{3-} in serum and urine. This coincides with clinical reports of decreased NO production in women with PrE attributed to the inhibition of NOS in the endothelial cells by endoglin through TGF-beta mediated pathway.[26,30]

The reduced NO bioavailability and the imbalance of several vasoregulatory factors in the PE group of animals displayed profound hypertension and significant deterioration of the renal functions demonstrated by increased serum urea and creatinine levels and manifest proteinuria. The kidney is considered a target organ in the pathophysiological process of PrE leading to glomerular endotheliosis that results in hypertension and proteinuria.[31]

Moreover, the PE group of animals showed significant increase in the anti-angiogenic molecule sFlt-1 a well-known etiologic factor of PrE. The increased sFlt-1

antagonizes new blood vessels formation in the placenta by binding to VEGF and placental growth factor (PlGF) and blocking their angiogeneic actions.[32] The serum levels of sFlt-1 exhibited inverse correlation with NO in the current study. This supports similar reports in patients and experimental models of PrE.[20,32] The combined effects of the increased sFlt-1 and declined NO production are anticipated to intensify the placental ischemia and excite the production of oxidative free radicles and vasoconstrictor molecules contributing in the pathogenesis of PrE.[34]

The lipid profile in the Con-P animals in the current study showed slight increasing trend in the serum TC and TG levels with normal LDL-C, HDL-C and LDL-C/HDL-C ratio. These findings were in agreement with previous reports of increased TC and TG in normal pregnant females that was attributed to the relative hyperestrogenemia and hyperinsulinism of pregnancy.[35,36] Unlike the control pregnant animals, the PE group of animals showed marked hyperlipidemic range of serum TC and TG and to a lesser extent LDL-C, together with decreased HDL-C levels and increased LDL-C/HDL-C ratio. These findings were in agreement with clinical reports of increased TC, TG, LDL-C in the first and the third trimester of pregnancy in patients with PrE.[37,38] Ardalic et al reported that hyperlipidemia in early pregnancy was associated with greater risk of PrE and persistent postpartum hypertension in the affected patients.[37]

The hyperlipidemia, increased LDL-C/HDL-C ratio and hypertension demonstrated in the PE group of animals receiving L-NAME treatment in the current study supported the recent findings of Aluko et al who reported that decreased NO levels shifts the serum lipoproteins levels towards an atherogenic profile in rats.[39] Furthermore, the blood lipids levels in the PE group of animals in the current study were forthrightly related to the clinical signs of PrE where TC, TG and LDL-C levels exhibited direct correlation with blood pressure, proteinuria, and sFlt-1 levels.

The coalitions of hyperlipidemia, and the lack of the vasodilator NO in the PE group of animals resulted in failure of development of the pups as manifested by low birth weight and high mortality rate. The deficiency of NO may in part leads to the vasospasm, decreased placental vasculogenesis, placental ischemia, defective blood flow and hypoxia of the developing fetus.[31]

The role of hyperlipidemia and decreased NO availability in hindering the growth and development of the fetuses in the PE group of animals was supported by the correlation analysis studies. The latter revealed that TG and LDL-C had inverse relationships with the number of live pups meanwhile TG levels were positively related to pup mortality rate. However, NO correlated directly with the number and weight of the pups.

The great impact of the blood lipids and NO levels on the growth and development of the fetus were well demonstrated in an in vitro study on the endothelial cells of the umbilical cord from pregnant females with physiological maternal hypercholesterolemia (PMH) (total cholesterol <280 mg/dl) and supra physiological maternal hypercholesterolemia (SPMH) (total cholesterol >280 mg/dl). The results showed that SPMH induced hypercholestrolemia in the fetal blood, decreased eNOS activity, increases arginase II activity. The decreased NO bioavailability generated atherosclerotic streaks in the umbilical arterial wall, and increased the intima/media ratio of the umbilical veins.[40] The latter changes induced endothelial dysfunction and vasospasm of the umbilical veins and decreased the blood flow associated with fetal growth abnormalities.[41]

Several therapeutic regimens have been tried in PrE, including antihypertensive drugs, calcium pump inhibitors, and corticosteroids that enhance lung maturity of the fetus. In addition to these drugs, $MgSO_4$ is very commonly used especially in patients with severe preeclampsia where it was found to be more effective than phenytoin or diazepam in reducing the risk of eclampsia, maternal deaths, and the recurrence of convulsions.[42,43] In the experimental model of PrE in rat, treatment by $MgSO_4$ reduced serum endoglin levels, blood pressure, proteinuria and increased the fetal birth weight.[20]

The role of NO in the establishment of the hemodynamic parameters during normal pregnancy was clearly illustrated in the PE-Mg group of animals in the current study. The administration of MgSO4 therapy resulted in significant stimulation of NO^{2-}/NO^{3-} production in the PE-Mg group of animals to greater extent in comparison to all the studied groups. The increased NO bioavailability was associated with manifest amelioration of the clinical signs of PrE including the hypertension, proteinuria, hyperlipidemia, renal dysfunctions and sFlt-1 levels. This was associated with better pregnancy outcome. The noteworthy increased pup weight in the PE-Mg group of animals linked to the increased NO levels reflected the enhancement of the vasculogenesis and the placental blood flow to the developing fetuses. This was in agreement with previous in vitro studies which showed that increased NO production in hypoxic human trophoblast cells stimulates the proangiogenic VEGF and PIGF and inhibits the anti-angiogenic sFlt-1 formation.[24,25,33] These findings confirmed earlier reports of improved clinical manifestations of PE through increasing NO production by vasodilators as sildenafil citrate, $MgSO_4$, dietary nitrate or NO precursors (l-arginine and L-citrulline).[20,44-46] There were some limitations associated with the current study including the failure of identification of the mechanism (s) through which the changes of nitric oxide levels influenced the blood lipid profile in the pregnant animals. An investigation of the effect of L-arginine- NO pathway on the genes expressions and the protein levels of the fatty acid metabolizing enzymes, the lipoproteins, and the nuclear receptors involved in lipid metabolism such as the

peroxisome proliferator-activated receptors (PPARs) in the normal pregnancy and preeclampsia. This need to be addressed in future researches where an additional experimental animal groups and in vivo experiments could be performed.

CONCLUSION

Decreased NO production in pregnant animals by blocking eNOS enzyme was associated with the classical signs of preeclampsia, hyperlipidemia, and poor pregnancy outcome demonstrated by high pup mortality rate, and low birth weight of the live pups. This could be attributed to the endothelial dysfunction induced by hypercholesterolemia, hypertriglyceridemia, increased LDL-C/HDL-C ratio and the vasospasm induced by lack of the endothelial relaxing action of NO, the increased levels of the vasoconstrictor molecules, and the anti-angiogenic molecules such as sFlt-1. All these factors interfere with normal blood vessels formation during placental angiogenesis leading to defective blood flow to the developing fetuses.

Increased NO production through $MgSO_4$ therapy was associated with decreased signs of PrE, hypolipidemia and increased pup birth weight and viability. This could be attributed to the vasodilator, antihyperlipidemic and antihypertensive effects of NO. It is to be concluded that preserving NO bioavailability and blood lipid homeostasis is crucial for the normal pregnancy outcome and prevention of preeclampsia. Routine regular biochemical assessments of the blood lipid profile and NO production in the pregnant females may be a helpful tool in early prediction of preeclampsia.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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