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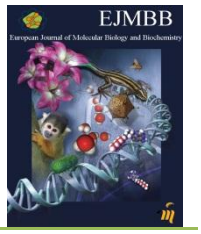

POSTPRANDIAL LIPEMIA AND POSTPRANDIAL ENDOTOXEMIA IN NORMAL PREGNANCY AFTER 20 WEEKS OF GESTATION (NP) AND PRE-ECLAMPSIA (PE)

Gaffar Sarwar Zaman

Assistant Professor, Department of Clinical Biochemistry, Government College of Medicine, King Khalid University, Abha, Kingdom of Saudi Arabia.

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ABSTRACT

To analyze postprandial lipemia and postprandial endotoxemia in normal pregnancy and pre-eclampsia. Materials and Methods: Fasting and postprandial total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides were determined sequentially in blood samples. Serum LPS concentrations were measured by endotoxin assay, based on a Limulus amebocyte extract. The endotoxin levels were determined in serum of normal pregnancy and pre-eclampsia at fasting, 1 hr., 2hr, 3 hr. and 4 hr. and their levels related with postprandial lipemia in the same conditions. Fasting and postprandial total cholesterol, LDL-cholesterol and triglycerides were increased more in pre-eclampsia than in normal pregnancy. Similarly endotoxin levels were also increased significantly in fasting and postprandial pre-eclamsia. The potential of the endotoxin in the development of an early-stage specific diagnostic biomarker for Pre-eclampsia is also emphasized.

INTRODUCTION

Pre-eclampsia (PE) affects approximately 3% of all pregnancies worldwide [1], with onset of symptoms in the late second or third trimester, most commonly after the 32nd week. It is characterized by blood pressure of 140/90 mm of Hg or rise in systolic blood pressure of more than 30mm of Hg or diastolic blood pressure of more than 15 mm of Hg after 20 weeks of gestation accompanied by proteinuria ≥ 300mg / 24 hrs. Or greater or equal to 1+ or 100mg /dl by dipstick response [2, 3]. Several studies have shown that endothelial dysfunction is related to hyperlipidemia [4, 5]. Altered lipid synthesis leading to decreased in PGI2: TXA2 ratio is also supposed to be an important way of pathogenesis in pregnancy induced hypertension [6]. Mild pre-eclampsia is defined as the presence of hypertension (BP > 140/90 mm Hg) on 2 occasions, at least 4 hours apart, but without evidence of end-organ damage in the patient [7].

Severe pre-eclampsia is defined as the presence of one of the following symptoms or signs in the presence of PE:

(1) Systolic blood pressure (SBP) of 160 mm Hg or higher and diastolic blood pressure (DBP) of 110 mm Hg or higher on 2 occasions at least 4 hours apart.
(2) Proteinuria of more than 5 g in a 24-hour collection or more than 3+ on 2 random urine samples collected at least 4 hours apart.
(3) Pulmonary edema or cyanosis.
(4) Oliguria (< 400 mL in 24 h).
(5) Persistent headaches.
(6) Epigastric pain and/or impaired liver function.
(7) Thrombocytopenia.
(8) Oligohydramnios decreased fetal growth, or placental abruption.

Insulin resistance is decreased ability of target tissues, such as liver, adipose, and muscle, to respond properly to normal circulating concentration of insulin.

Corresponding Author

Dr. Gaffar Sarwar Zaman
Email: - drgszaman@gmail.com

Endothelial injury is a key factor in the pathogenesis of the disease [8]. Studies have shown that lipids accumulate in arterial intima cells and in macrophages causing endothelial injuries [9]. Bacterial endotoxin [lipopolysaccharide (LPS)], a component of the Gram-negative bacteria cell wall that is present in large quantities in the human gut [10]. Endotoxins circulate in the plasma of healthy human subjects at low concentrations (known as metabolic endotoxemia), and an elevated concentration of circulating LPS has been associated with a higher risk for atherosclerosis [11]. There is evidence that metabolic plasma LPS levels are modulated by food content: the higher the fat content, the higher the concentration of plasma LPS [12] (11). Small amounts of LPS are absorbed from the gut in healthy animals [13], and there is evidence that chylomicrons likely also transport significant amounts of absorbed gut LPS [14-16]. In concordance with these data, some studies have shown that a high-fat meal leads to an increase in postprandial endotoxemia. Metabolic endotoxemia could be involved in the development of PE, since it has been associated with the development of development of obesity-related co-morbidities [17, 18].

Thus, the aim of this study was to analyze postprandial endotoxemia and compare it in normal pregnancy (NP) and PE and to determine its relationship with postprandial lipemia.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of the hospital, and all women gave written informed consent. Prior to the study, participants were informed that their confidentiality would be maintained and consent was obtained. For the group of NP, individuals selected had to be healthy, between 18 and 45 years old, premenopausal, non-hypertensive, non-diabetic, not pregnant, not obese [body mass index (BMI) < 30 kg/m2], and could not be taking medication known to affect bone and lipid metabolism or e taking vitamin, mineral, or phytoestrogen supplements. Women for the PE group had to be in the same age group, previously non-hypertensive, non-diabetic, could not be obese (BMI < 30 kg/m2), could not be receiving estrogen replacement therapy or any other medication known to affect bone and lipid metabolism or be taking vitamin, mineral, or phytoestrogen supplements. None of the women smoked. On the morning of the visit, blood pressure, weight, and height were measured and compliance with dinner instructions was verified with a questionnaire. After that, each participant underwent a structured examination, which included an interview. Height, weight, waist circumference (WC) and hip measurements, a fasting venipuncture, and sequential determination of serum lipids were done. Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. BMI was calculated as weight (kilogram) divided by height (in meter) squared. WC was determined to the nearest 0.1 cm using a measuring tape positioned at the midpoint between the lowest rib and the iliac crest and hips were measured at the largest gluteal circumference. These measurements were used to calculate the waist-to-hip ratio (WHR). Then, blood pressure was measured using a standard mercury sphygmomanometer. Samples were centrifuged; serum was collected and stored at 20 °C until analyzed. Lipid profiles comprising TC, HDL-C, LDL-C, and TG concentrations were measured at fasting and at 1, 2, 3, and 4 h post-load. Sample serum concentrations of TC, HDL-C, and TG were measured by enzymatic colorimetric methods using a Star Plus 21 semi-autoanalyser of Rapid Diagnostic Company. Calculation of LDL-C concentrations was based on the Friedewald equation [19]. The diagnosis of DM was based on WHO criteria [20], i.e. a fasting plasma glucose level > 7.0 mmol/L or > 126 mg/dL, or a 2-h postprandial plasma glucose level > 11.1 mmol/L or > 200 mg/Dl on more than one occasion, with symptoms of diabetes.

The endotoxin concentration in a sample was measured using the Pierce LAL Chromogenic Endotoxin Quantitation Kit via a chromogenic signal generated in the presence of endotoxins. Internal control of recovery calculation was included in the assessment. All samples were tested in duplicate. Samples can be measured on a microplate absorbance reader at 405nm. A standard curve is created using the E. coli endotoxin standard included with each kit to calculate endotoxin levels as low as 0.1 EU/mL, where one endotoxin unit/mL (EU/mL) equals approximately 0.1ng endotoxin/mL of solution. The endotoxin content was expressed as endotoxin units (EU) per mL. Exhaustive care was taken to avoid environmental endotoxin contamination and all material used for sample preparation and the test was pyrogen-free.

All data were entered into an Excel spreadsheet, and were analysed using standard statistical software such as SPSS. Chi-square test was used for categorical variables. All numerical data were presented as mean ± standard deviation. A P value of less than 0.05 was considered statistically significant.

RESULTS

The result of the study on the relationship between the serum lipids in NP and PE are represented in Figures 1–4. The mean TC in mg/dL was 177, 188, 192, 168, and 166 at fasting, 1, 2, 3, and 4 h in the NP vs. 192, 199, 208, 210, and 193 in the PE during the same duration. Cholesterol concentrations showed a significant reduction after 2 h, to reach values similar to the baseline after 4 h in NP but not in PE. The mean HDL-C in mg/dL was 49, 46, 44, 43 and 44 at fasting, first, second, third and fourth hours after the test meal in the NP vs. 44, 41, 43, 44, and 40 in the PE during the same time interval. This shows that HDL-C concentration was decreased more in PE compared to NP but it was not significant. The mean LDL-C in mg/dL was 128, 137, 126, 118, and 102 at fasting, 1, 2, 3, and 4 h in the NP vs. 164, 173, 186, 178, and 164 in the PE during the same amount of time.

The triglyceride concentration (in mg/dL) was in the normal range (87, 92, 93, 90 and 89) in NP but showed a higher level (139, 158, 168, 142 and 137) in PE. The endotoxin level at fasting, 1 hr., 2 hr., 3 hr., 4 hr. in NP women was 0.33, 0.39, 0.47, 0.51 and 0.52 respectively. But the endotoxin level showed a marked increase in PE and the levels observed were 0.55, 0.58, 0.69, 0.59 and 0.57. The difference was highly significant (P<0.05).

Figure 1. The mean total cholesterol in mg/dl at fasting, 1, 2, 3 and 4 hours in Normal Pregnancy and Pre-Eclampsia

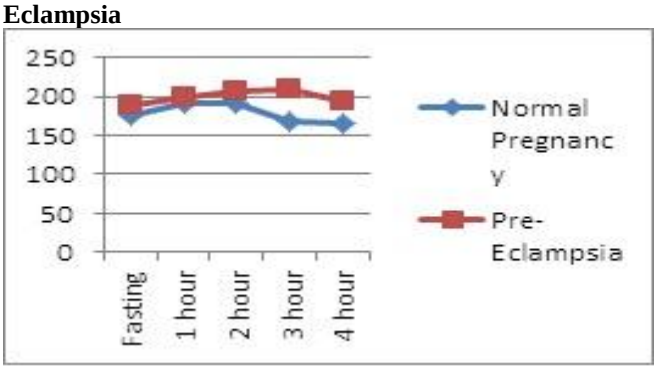


Figure 2. The mean HDL cholesterol in mg/dl at fasting, 1, 2, 3 and 4 hours in Normal Pregnancy and Pre-Eclampsia

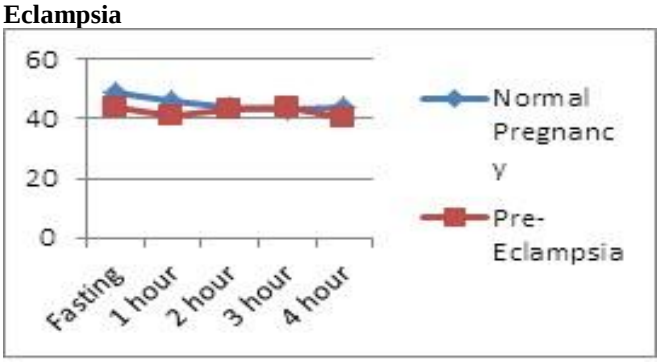


Figure 3. The mean LDL cholesterol in mg/dl at fasting, 1, 2, 3 and 4 hours in Normal Pregnancy and Pre-Eclampsia

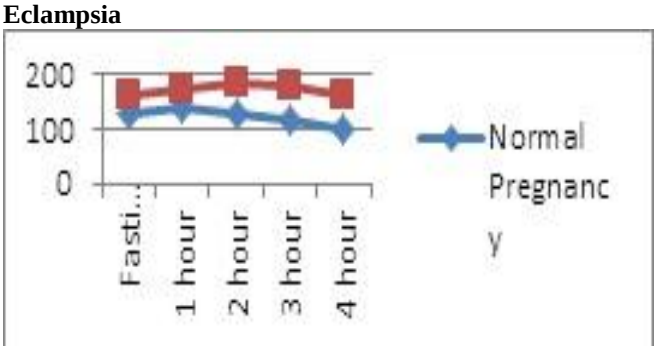


Figure 4. The mean Triglyceride concentration in mg/dl at fasting, 1, 2, 3 and 4 hours in Normal Pregnancy and Pre-Eclampsia

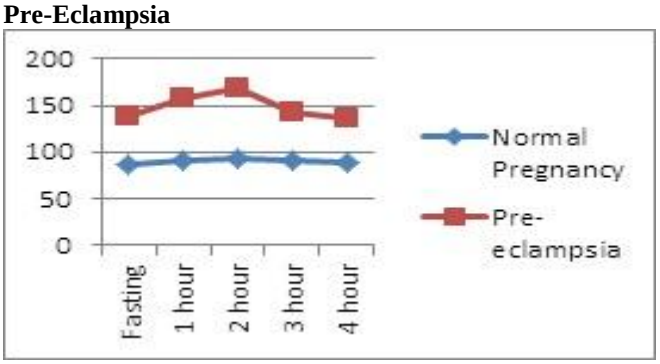
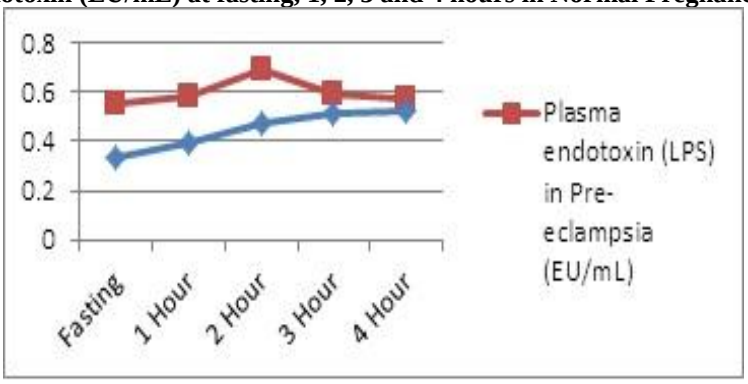


Figure 5. The Plasma Endotoxin (EU/mL) at fasting, 1, 2, 3 and 4 hours in Normal Pregnancy and Pre-Eclampsia



DISCUSSION

Lipid metabolism is altered during pregnancy and is characterized by normal or even low cholesterol during early pregnancy and hypertriglyceridaemia in late pregnancy. The anabolic phase of early pregnancy produces metabolic changes that encourage lipogenesis and fat storage in preparation for the catabolic phase of late pregnancy in which there is rapid fetal growth. The insulin resistance of pregnancy increases lipolyis in adipose tissue, leading to an enhanced flux of fatty acids to the liver. This promotes the synthesis of very low density lipoproteins (VLDL) and as a result, increased triglyceride concentrations. In addition, insulin resistance reduces the activity of lipoprotein lipase an insulin dependent enzyme that is responsible for VLDL clearance from plasma. Therefore, VLDL remains in the plasma for longer and ultimately leads to accumulation of low density lipoprotein (LDL) [21]. Some studies have shown that the most

dramatic damage in the lipid profile of pregnancy is serum hypertriglyceridaemia which is even higher in toxaemia of pregnancy [22]. The finding of hypertriglyceridaemia is in keeping with the findings of Enquobahrie and Cekmen [23].

Several clinical studies have shown that the magnitude and duration of Post-Prandial Lipemia (PPL) are positively related to the pathogenesis and progression of coronary heart disease (CHD). Postprandial lipid metabolism refers to the series of metabolic events that occur following the ingestion of a meal containing fat. Dietary fat is principally composed of TG; PPL therefore being characterized by an increase in plasma TG concentration [24]. PPL is influenced by various parameters such as gastric emptying time, intestinal absorption, and lipoprotein lipase activity. Some studies have shown that the gastric emptying of liquids and solids decreases with age [25], but intestinal motility is not altered with age [26]. Pancreatic secretion slightly decreases with age [27]. However, Arora *et al* [28, 29]. Studying healthy individuals have reported that fecal excretion, and, consequently, fat absorption changes slightly with age, suggesting that the decrease in pancreatic secretion is not enough to hinder the normal digestive process. One could imagine that because older individuals have a longer gastric emptying time, the absorption of fat would be slowed, justifying a late elevation in triglyceridemia. With age, gastric emptying rate and lipoprotein lipase activity are known to decrease, and a reduction of pancreatic lipase secretion and a delay in the clearance of TG-rich lipoproteins have also been observed. Bibliographical data on postprandial metabolism in NP and PE women are scarce and the studies that have been undertaken involve very small numbers of subjects. It is also difficult to compare data due to of the variety of food employed in the different studies. The lower PPL displayed by the NP in this study was also found in other studies, with levels of TG for NP and PE similar to our data [30]. Nabeno *et al* [31] reported similar results, but differ in the baseline characteristics of the women studied and in the food consumed, which was given as a fat-rich cream.

The present study showed significantly increase in TG levels in sera of pre-eclampics compared with control and may be attributed to the principle modulator of hypertriglyceridemia oestrogen as pregnancy is associated with hyperoestrogenaemia. Oestrogen induces hepatic biosynthesis of endogenous triglycerides, which is carried by VLDL-C [32]. Increased TG levels results in endothelial cell dysfunction and in pre-eclampsia gets deposited in predisposed vessels [33], causes generation of small dense LDL [34] and hypercoaguability. Kornacki J et al [35] who found an elevated serum triglycerides as an important contributing factor of pre-eclampsia. Also the present study showed insignificant difference in TC, LDL, and HDL in sera of pre-eclamptic groups compared with control groups. These results were consistent with the results reported by Kashinakunti SV et al. [36] who observed an insignificant difference in TC, LDL, HDL parameters of cases and controls. Many of the variations in the lipid profile values between different populations can be attributed to disparate environmental factors and dietary habits. Thus, these variations may contribute to the pathogeneses of pre-eclampsia.

Excessive LPS absorption, however, could evidently be harmful and could lead to acute or chronic inflammation. Increased LPS absorption, for example, could exacerbate the risk for several chronic diseases, such as alcoholic liver injury [37] nonalcoholic steatohepatitis, HIV/AIDS, and inflammatory bowel disease [38]. In theory, dietary fat could increase LPS absorption in several ways. One way would be through promotion of paracellular uptake of macromolecules as a result of deleterious effects of fatty acids on tight-junction integrity [39]. In alternative mechanism explaining fatty-acid dependent LPS absorption involves internalization of LPS by the enterocyte, followed by association of some of the internalized LPS with chylomicrons and concomitant basolateral secretion of LPS with the chylomicrons or by association of independently transcytosed LPS with newly released chylomicrons. In conclusion, LPS levels were higher in PE than those of normal pregnancy. In consequence, it has been hypothesized that endogenous LPS levels could be responsible for the low-grade inflammation observed in PE.

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