

Serum Lipid Levels, Atherogenic Indices and Alkaline Phosphatase Activity in Normal Pregnancies

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Abstract: Changes in maternal lipids and alkaline phosphatase (ALP) activity occur during normal pregnancy. There is scarce data in regards to atherogenic indices and relationship of lipids with ALP during pregnancy. The study therefore aimed at determining serum lipid levels, atherogenic indices, and ALP activity as well as their relationship in normal pregnancies. The case-control study compared the parameters of second (n=37) and third (n=40) trimester pregnancies with normal pregnant controls (n=40) aged 18-35 years. Results showed a significant ($P<0.0005$) rise in the levels of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), triglycerides (TGs), non-high density lipoprotein cholesterol (nHDL-C) and ALP in the pregnant women compared to anthropometrically matched non-pregnant women. A significant ($P<0.02$) rise in Castelli's risk index (CRI-1), atherogenic coefficient (AC), TG/HDL-C, atherogenic index of plasma (AIP) was observed in the pregnant women compared to the matched controls. Pearson correlation showed a positive correlation of gestation age with ALP ($r=0.365$), TC ($r=0.450$), HDL-C ($r=0.311$), VLDL-C ($r=0.338$), TGs ($r=0.338$), and nHDL-C ($r=0.291$). A positive correlation of ALP was observed with TC ($r=0.689$), LDL-C ($r=0.608$), VLDL-C ($r=0.231$), TGs ($r=0.231$), and nHDL-C ($r=0.647$). The atherogenic indices neither correlated with gestation age nor ALP. The present study shows that serum lipids increase in association with ALP during normal pregnancy. We recommend further studies in women with complicated pregnancies to gain insights into the patho-physiology of the association.

Keywords: cholesterol, pregnancy, alkaline phosphatase, lipoproteins, atherogenic indices, triglycerides.

INTRODUCTION

Physiologic pregnancy is accompanied with metabolic adaptations which could influence the metabolism of lipids and lipoproteins. Observational and epidemiological studies suggest that maternal atherogenic lipid profile may predispose to complications during and after pregnancy, both for the fetus and the mother. The onset of dyslipidemia during pregnancy is an important risk factor for future cardiovascular disease (CVD) in the mother and child, preeclampsia, gestational diabetes mellitus, fetal macrosomia, preterm birth [1-5]. On the contrary, intrauterine growth retardation (IUGR) has been associated with decreased maternal lipid transfer to the fetus either in normal placenta or secondary to placental abnormalities in pregnant women [6, 7].

In searching for new cardiovascular risk factors relating to lipids and lipoproteins, several lipoprotein ratios or atherogenic indices have been defined [8, 9]. Castelli's risk index (CRI-1 and CRI-2), atherogenic coefficient (AC), TG/HDL-C (high density lipoprotein cholesterol) ratio and atherogenic index of

plasma (AIP) reflect the metabolic and clinical interactions between lipid fractions. These lipid ratios are useful in predicting plasma atherogeneity especially when the routine lipid profile becomes difficult to interpret [9, 10]. It has been shown that the measurement of non (n)HDL-C is a better predictor of CVD than low density lipoprotein cholesterol (LDL-C) [11]. The use of nHDL-C for CVD risk prediction has been emphasized in several guidelines and consensus papers [9]. nHDL-C is determined as a valid surrogate to apolipoprotein B 100 (apolipoprotein of LDL-C and triglyceride (TG)-rich lipoprotein) in the assessment of atherogenic cholesterol and lipoprotein burden [11]. Therefore, their applications in interpreting hyperlipidaemia of pregnancy cannot be overemphasized.

Alkaline phosphatases (ALPs) are a group of ubiquitous membrane-bound glycoproteins that hydrolyse a broad range of monophosphate esters at alkaline pH optima [12]. Other than liver, bone, bile duct, kidney, intestinal mucosa and placenta, ALP is expressed in adipose tissue which regulates intracellular

fat deposition in preadipocytes during adipogenesis [13], suggesting a role of ALP in lipid metabolism. Another study predicted a link between ALP activity and insulin secretion/action from the pancreatic beta cells [14]. This enzyme is also known to participate in transfer of glucose and fatty acids across the cell membrane [15]. The precise metabolic function of the enzyme is still unclear. During pregnancy serum total ALP levels begin to rise at the fourth month of gestation, increasing progressively through the second and third trimesters [16]. This maternal elevation reflects the placental isoenzyme emanating from the trophoblastic cells of the developing placenta. Placental (PALP) during pregnancy has a role in division of normal and transformed cells [17]. Extreme elevation of serum ALP in pregnancy may be an indicator of placental insufficiency, IUGR and low birth weight [18]. Studies suggest a possible association between increased PALP levels and pre-eclampsia [19].

Studies have separately described changes in the concentrations of lipids [20, 21] and ALP activity [16] during normal pregnancy. Only one study has attempted to correlate serum lipids with ALP activities during normal pregnancy. It is in this regard that the present study investigated serum lipids, atherogenic indices, ALP activity in normal, second and third trimester pregnancies as well as their inter-relationship during pregnancy.

MATERIALS AND METHODS

Study Design

This case-control study compared serum lipid levels, atherogenic indices, ALP in second (N=37) and third (N=40) trimester normal pregnancies with age matched non-pregnant women (N=40).

Study Area and Population

A sample of female participants aged 18-35 years, were randomly drawn from women attending the ante-natal clinic at the Federal Medical Centre Makurdi, Nigeria from January to March 2018. Anthropometrically matched female controls were obtained from female patients attending the same hospital for general check up within the same period.

Selection Criteria

Individuals were eligible to participate in the study if they: (a) were within the reproductive age of 18 to 35 years; (b) had no history of hypertension and were not using antihypertensive medications; (c) were free of any other major systemic illnesses (e.g. liver disease, cancer, diabetes mellitus); (d) were free from pregnancy complications; (e) were non-smokers. All subjects were availed with informed consent, and the study was approved by the institutional ethical committee.

Data Collection

Participants provided information on their demographic characteristics, detailed medical history, dietary and lifestyle habits. All participants were required to fast for 12 hours before intravenous blood sample collection for biochemical determinations. Physical examination was carried out by trained staff and physicians using standard protocols.

Body mass index (BMI)

Body weight and height were measured with the subject barefoot and wearing light clothing. Body weight to the nearest 0.1 kg and height to the nearest centimeter were measured and BMI was calculated as weight (kilograms)/height (meters squared).

Blood pressure

Systolic and diastolic blood pressure (SBP and DBP) of the participants was measured twice in seated position after a 5-min rest using a mercury sphygmomanometer.

Determination of biochemical parameters

Participant's blood samples were collected into plain vacutainer tubes and centrifuged at 3000 rpm for 10 minutes within 1 hour of blood collection. Serum was used for the determination of levels of total cholesterol (TC), TGs, HDL-C, ALP using Randox reagent kits (Randox Laboratories Ltd., County Antrim, UK) on a spectrophotometer (Optima SP-300 Spectrophotometer; Optima INC. Tokyo, Japan) immediately after separation. Friedewald's equation was used in estimating very LDL-C (VLDL-C) and LDL-C [22]. nHDL-C was calculated as the difference between TC and HDL-c [23]. The atherogenic indices were calculated as follows; CRI-1=TC/HDL, CRI-2=LDL/HDL, AC=(TC-HDL)/HDL, TG/HDL ratio and AIP=log (TG/HDL) [10].

Statistical analysis of data

Data were presented as means and standard deviations for continuous variables. Analysis of variance was used for between-group assessments followed by least significant difference *post hoc* honestly significant difference test. Pearson's correlation coefficient was used to examine the correlation between lipids and gestation age, ALP and lipids. All statistical analyses were performed using the IBM Armonk, NY, USA, SPSS version 21. A two-sided P<0.05 was considered statistically significant.

RESULTS

Table-1 shows the mean of anthropometric indices in non-pregnant, second and third trimester pregnancies. A significant increase in BMI was observed in the second ($P=0.018$) and third ($P=0.000$) trimester pregnancies compared to non pregnant females; where as a non significant ($P>0.05$) difference in BMI was observed between the second and third

trimester pregnancies. A non significant ($P>0.05$) difference in maternal age and blood pressure was observed between the three groups.

Table-2 shows the mean levels of serum lipids and ALP in non-pregnant, second and third trimester pregnancies. A significant ($P<0.000$) change in ALP, TC, VLDL-C, TGs, and non-HDL-C levels were observed in the three groups compared; with a post hoc test showing a significant ($P<0.002$) increase in the levels of ALP, TC, VLDL-C, TGs, non-HDL-C in the second and third trimester pregnancies compared to non pregnant women. Post hoc analysis also revealed a significant ($P<0.02$) raised ALP, TC, LDL-C, VLDL-C, TGs, and non-HDL-C levels in third trimester pregnancy compared to the second trimester pregnancy, where as a non significant ($P>0.05$) difference was observed in LDL-C levels of second trimester pregnancy compared to non pregnant women. No significant ($P>0.05$) change was observed in HDL-C level when the three groups were compared.

The mean atherogenic indices in non-pregnant, second and third trimester pregnancies are presented in table 3. A significant ($P<0.05$) change was observed in the atherogenic indices when the three groups were compared. A post hoc test revealed a significant ($P<0.02$) increase in CRI-1, TG/HDL, AC, AIP of second and third trimester pregnancies compared to non

pregnant women, while a significant ($P=0.012$) increase in CRI-2 was observed only in the third trimester compared to first trimester pregnancy. No significant ($P>0.05$) difference was observed between the atherogenic indices of the second and third trimester pregnancies.

Figure-1 shows the Pearson correlation of ALP with gestation age. A significant positive correlation ($P=0.001$, $r=0.365$) was observed between ALP and gestation age in the pregnant women studied.

Figure-2 shows the pearson correlation of serum lipids with gestation age. A significant positive correlation was observed between TC and gestation age ($P=0.000$, $r=0.450$), HDL-C and gestation age ($P=0.006$, $r=0.311$), nHDL-C and gestation age ($P=0.01$, $r=0.291$), VLDL-C and gestation age ($P=0.003$, $r=0.338$), TGs and gestation age ($P=0.003$, $r=0.338$).

The Pearson correlation of serum lipids with ALP in the pregnant women studied is presented in figure 3. A significant positive correlation was observed between TC and ALP ($P=0.000$, $r=0.689$), nHDL-C and ALP ($P=0.000$, $r=0.647$), LDL-C and ALP ($P=0.000$, $r=0.608$), VLDL-C and ALP ($P=0.043$, $r=0.231$), TGs and ALP ($P=0.043$, $r=0.231$).

Table-1: Age, BMI, blood pressure in non-pregnant, second and third trimester pregnancies

Anthropometry	Non pregnant (NP) n=40		Second trimester (ST) n=37		Third trimester (TT) n=40		F-value	P-value
	NP	ST	P	NP	TT	P		
AGE years	29.05±8.57	27.43±4.62		29.03±4.61	0.833		0.437	
BMI kg/m ²	25.22±3.54	27.80±6.35		29.32±3.99	7.663		0.001*	
SBP mmHg	109.08±6.42	109.19±11.40		106.65±10.33	0.885		0.416	
DBP mmHg	69.15±6.91	69.59±9.89		69.0±9.82	0.045		0.956	
Post Hoc	NP	ST	P	NP	TT	P	ST	TT
AGE	29.05±8.57	27.43±4.62	0.663	29.05±8.57	29.03±4.61	0.65	27.43±4.62	29.03±4.61
BMI	25.22±3.54	27.80±6.35	0.018*	25.22±3.54	29.32±3.99	0.00	27.80±6.35	29.32±3.99
SBP	109.08±6.42	109.2±11.40	0.958	109.08±6.42	106.65±10.33	0.26	109.2±11.40	106.65±10.33
DBP	69.15±6.91	69.59±9.89	0.828	69.15±6.91	69.0±9.82	0.94	69.59±9.89	69.0±9.82

*significant difference, Body mass index- BMI, systolic blood pressure-SBP, diastolic blood pressure-DBP,

Table-2: Lipid profile, alkaline phosphatase in non-pregnant, second and third trimester pregnancies

Parameters	Non pregnant (NP) n=40	Second trimester (ST) n=37	Third trimester (TT) n=40	F-value		P-value			
Post Hoc	NP	ST	P	NP	TT	P	ST	TT	P
TC mmol/L	4.23±0.62	4.91±1.08	0.001*	4.23±0.62	5.83±0.98	0.000*	4.91±1.08	5.83±0.98	0.000*
HDL-C mmol/L	1.49±0.35	1.46±0.62		1.49±0.35	1.66±0.64	1.470		1.66±0.64	0.234
LDL-C mmol/L	2.30±0.68	2.66±0.76		2.30±0.68	3.17±1.13	9.873		3.17±1.13	0.000*
VLDL-C mmol/L	0.48±0.19	0.80±0.27		0.48±0.19	1.00±0.32	38.911		1.00±0.32	0.000*
TG mmol/L	0.97±0.30	1.76±0.61		0.97±0.30	2.21±0.71	49.378		2.21±0.71	0.000*
nHDL-C mmol/L	2.75±0.68	3.46±0.87		2.75±0.68	4.17±1.09	25.252		4.17±1.09	0.000*
ALP IU/L	55.9±18.6	84.4±25.9	0.000*	55.9±18.6	117.0±42.9	38.962		117.0±42.9	0.000*
CRI-1	3.02±0.85	3.98±1.73	0.009*	3.02±0.85	4.16±1.90	6.065		4.16±1.90	0.003*
CRI-2	1.70±0.77	2.24±1.27	0.069	1.70±0.77	2.45±1.68	3.516		2.45±1.68	0.033*
AC	2.02±0.85	2.96±1.70	0.009*	2.02±0.85	3.16±1.90	6.065		3.16±1.90	0.003*
TG/HDL	0.71±0.32	1.58±1.13	0.000*	0.71±0.32	1.58±0.82	15.022		1.58±0.82	0.000*
AIP	-0.19±0.18	0.11±0.28	0.000*	-0.19±0.18	0.14±0.23	23.829		0.14±0.23	0.000*

*significant difference, high density lipoprotein cholesterol-HDL-C, low density lipoprotein cholesterol-LDL-C, very low density lipoprotein cholesterol-VLDL-C, triglycerides-TGs, non- high density lipoprotein cholesterol-nHDL-C

Table-3: Atherogenic indices in non-pregnant, second and third trimester pregnancies

Atherogenic indices	Non pregnant (NP) n=40	Second trimester (ST) n=37	Third trimester (TT) n=40	F-value		P-value			
Post Hoc	NP	ST	P	NP	TT	P	ST	TT	P
CRI-1	3.02±0.85	3.98±1.73	0.009*	3.02±0.85	4.16±1.90	0.001*	3.98±1.73	4.16±1.90	0.573
CRI-2	1.70±0.77	2.24±1.27	0.069	1.70±0.77	2.45±1.68	0.012*	2.24±1.27	2.45±1.68	0.499
AC	2.02±0.85	2.96±1.70	0.009*	2.02±0.85	3.16±1.90	0.001*	2.96±1.70	3.16±1.90	0.573
TG/HDL	0.71±0.32	1.58±1.13	0.000*	0.71±0.32	1.58±0.82	0.000*	1.58±1.13	1.58±0.82	0.994
AIP	-0.19±0.18	0.11±0.28	0.000*	-0.19±0.18	0.14±0.23	0.000*	0.11±0.28	0.14±0.23	0.545

*significant difference, Castelli's risk index (CRI-1 and CRI-2), atherogenic coefficient (AC), TG/HDL-C (triglycerides/high density lipoprotein cholesterol ratio) and atherogenic index of plasma (AIP)

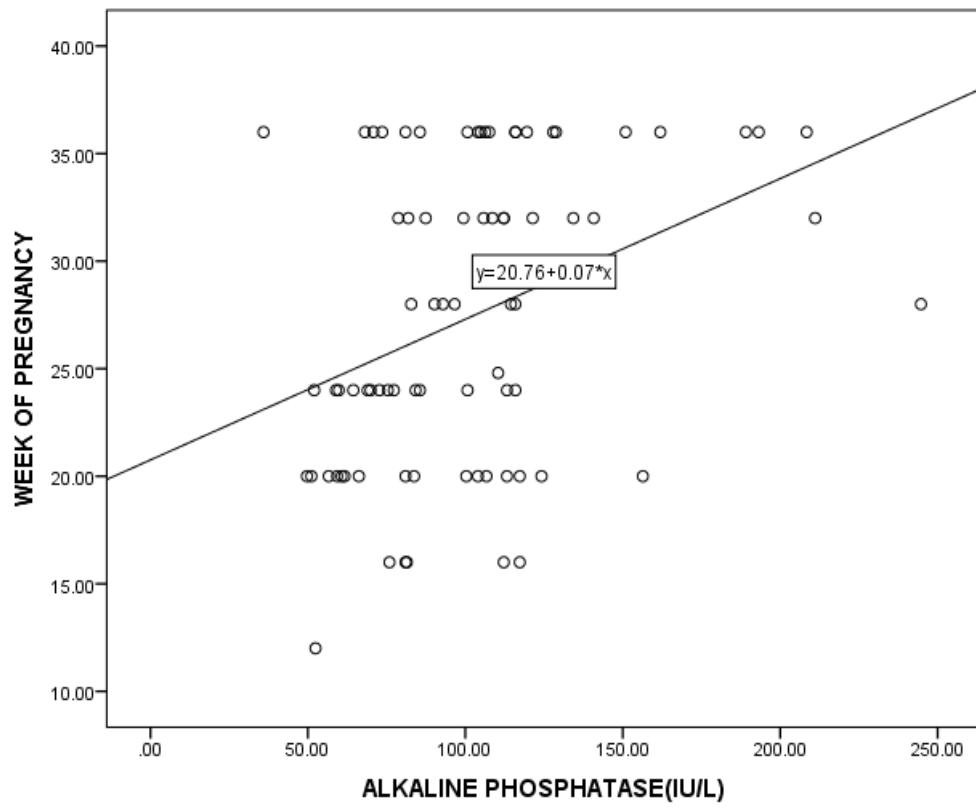


Fig-1: Correlation graph of gestation age with alkaline phosphatase in pregnant women

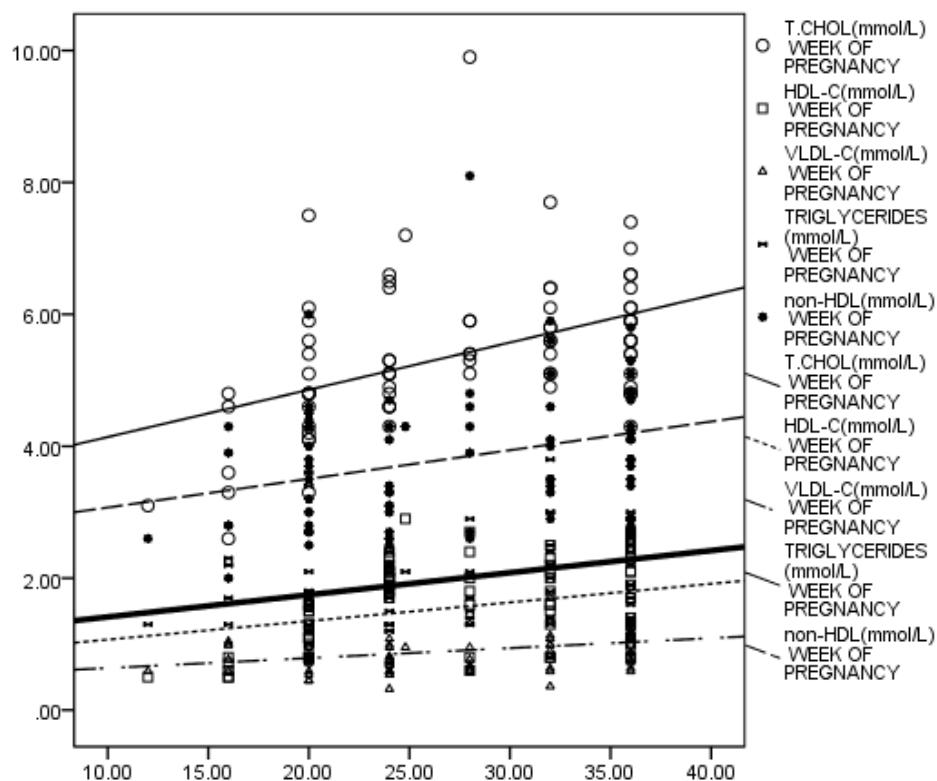


Fig-2: Correlation graph of gestation age with serum lipids in pregnant women

N=77, TC and week of pregnancy ($P=0.000$, $r=0.450$), HDL-C and week of pregnancy ($P=0.006$, $r=0.311$), nHDL-C and week of pregnancy ($P=0.01$, $r=0.291$), VLDL-C and week of pregnancy ($P=0.003$, $r=0.338$), TGs and week of pregnancy ($P=0.003$, $r=0.338$).

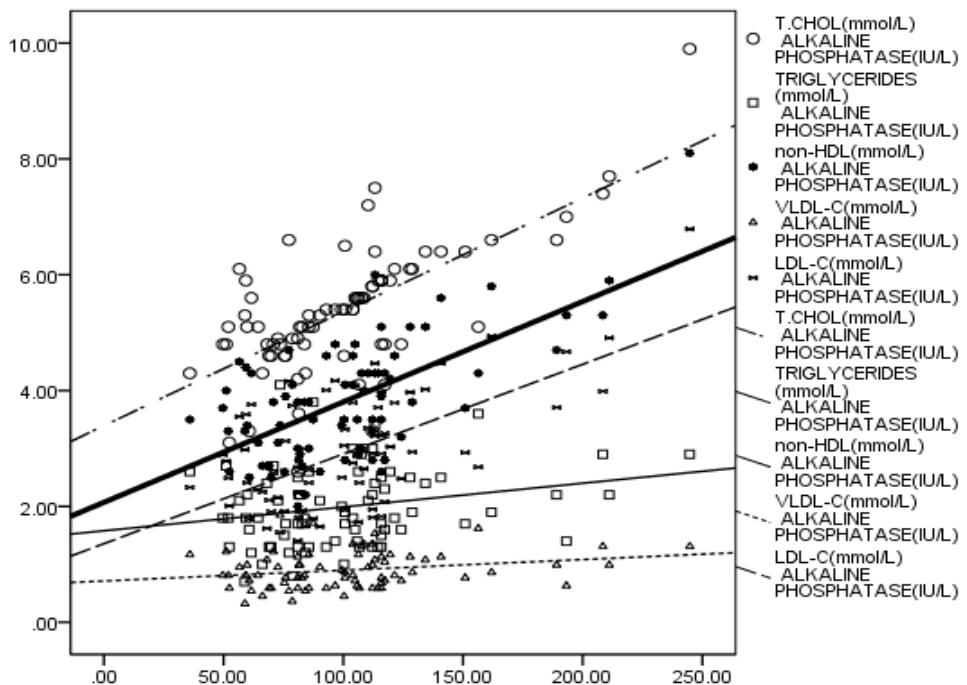


Fig-3: Correlation graph of alkaline phosphatase with serum lipids in pregnant women

N=77, TC and ALP ($P=0.000$, $r=0.689$), nHDL-C and ALP ($P=0.000$, $r=0.647$), LDL-C and ALP ($P=0.000$, $r=0.608$), VLDL-C and ALP ($P=0.043$, $r=0.231$), TGs and ALP ($P=0.043$, $r=0.231$).

DISCUSSION

Maternal dyslipidemia is associated with risk of developing pregnancy complications [2]. Atherogenesis enhanced by hyperlipidemia is reported in some epidemiologic and biochemical studies as a diffuse process involving the entire arterial tree that begins earlier before birth [24]. Lipid lowering interventions may be required in dyslipidemic mothers to decrease atherogenesis in their children [25].

The present study showed an increase in TC, LDL-C, nHDL-C, VLDL-C, and TGs levels in the pregnant women compared to the non pregnant women. No change was observed in the HDL-C levels of the pregnant and non pregnant women. The positive correlation observed between serum lipids and gestation age shows that as pregnancy progresses TC, HDL-C, nHDL-C, VLDL-C, and TGs levels simultaneously increased.

Most pregnant women have been shown to develop elevated plasma lipid levels that increase as the pregnancy progresses [26-29]. The results of this present study were similar to that of Lippi *et al.*, Choi & Pai., Diareme *et al.*, Okojie *et al.*, Phuse SS *et al.*, Husain *et al.* Saarelainen *et al.*, Lippi *et al.*, evaluated lipids and lipoproteins in 57 women at different gestational ages; they observed a significant increase in TC, HDL-C, LDL-C, TGs in the second and third trimester pregnancies compared to the non pregnant controls. An increase in the serum lipids were also observed as the gestation age progresses [30]. Choi & Pai observed a gradual increase in serum TGs, TC, and

LDL-C as pregnancy proceeded [31]. Diareme *et al.*, showed a significant increase in TC, HDL-C, LDL-C, TGs, in second and third trimester pregnancies compared to non pregnant women with a positive correlation observed between week of pregnancy with TC, HDL-C, LDL-C, TGs, TC/HDL, LDL/HDL [32]. A lipid profile study conducted in 120 pregnant women during normal gestation (40 women in each trimester) and 40 non-pregnant, healthy women as control by Okojie *et al.*, showed elevated levels of TC, HDL-C, LDL-C, TGs in the first, second and third trimesters compared to non-pregnant controls [21]. In a study conducted by Phuse SS *et al.*, on 75 pregnant women between 24-35 years of age across each trimester of pregnancy against a control group of 70 non-pregnant women, lipid profile changes were observed. This study showed that the serum concentrations of TC, LDL-C, VLDL-C and TGs increased from second trimester to third trimester while that of HDL-C decreased as compared to that of the control group [33]. A study conducted by Husain *et al.*, showed that a mother's serum cholesterol concentrations rise by approximately 50-70% during pregnancy compared to normal concentrations [29]. Saarelainen *et al.*, recorded a significant increase of serum TC, LDL-C, HDL-C and TGs during pregnancy when compared with the non-pregnant state and towards the end of pregnancy [34].

A controversial pattern of HDL-C changes has been reported by other previous studies. The present study observed a non significant change in HDL-C level when pregnant and non pregnant women were compared; however, Pearson correlation results showed

a positive relationship between HDL-C and gestation age. In consonance with the present study Kar & Sinha did not find any significant alteration of HDL in normotensive pregnant mothers compared with non pregnant females [19]. Loke *et al.*, showed increases in HDL-C level during the second trimester but a decrease during the third trimester [35]. Pusukuru *et al.*, observed a decrease in HDL-C as pregnancy progressed from second trimester to third trimester [36]. Several studies [27, 29, 30, 32, 34, 37] have shown a progressive increase in HDL-C as pregnancy advanced, which is in consonance with the correlation results of HDL-C with gestation age in the present study. Studies have linked the progressive raise in HDL-C in the pregnant mother as a protective measure to offset elevations in atherogenic LDL-C and TG levels in normal pregnancies [38]. The varying pattern of HDL-C changes reported by several studies could be due to variation in the nature (normal, complicated, multiparous and primiparous pregnant women) of the sample population. Multiparous women tend to have a relative decrease in HDL-C levels in comparison to their primiparous counterparts [37]. Derangements of elevated cholesterol fractions with lower HDL-C levels appear to be more pronounced in women with gestational hypertension, diabetes, and preeclampsia [38, 39].

In this present study, changes in atherogenic indices were also observed when a comparison was made between the pregnant and non pregnant women. However, no correlation was observed between the atherogenic indices and gestation age. Similar to this present study Lippi *et al.*, observed a significant increase of AIP in the second and third trimester pregnancies compared to the non pregnant controls, while total to HDL-C ratio was significantly increased only in third trimester compared to non pregnant controls [30].

The present study observed a progressive increase in serum ALP levels from non pregnant levels through second trimester to third trimester pregnancies. A positive correlation was observed between serum ALP and lipids; TC, LDL-C, nHDL-C, VLDL-C, and TGs levels increased along with serum ALP. This observation is similar to the study of Choi & Pai who observed a positive correlation of TC and TGs with ALP in normal pregnant women [31]. Choi & Pai showed that serum ALP activity averaged 2.1-fold higher in the late third trimester than in the first trimester. The present study however showed no correlation between the atherogenic indices and ALP. The elevation of ALP activity in maternal serum during late pregnancy has been well documented [40] and primarily reflects the presence of placental ALP in the maternal circulation [41].

One of the maternal metabolic adjustments during pregnancy is accumulation of fat depots in maternal tissues [42]. During this anabolic phase, the number of insulin receptors on the adipocytes increases, culminating into increased insulin sensitivity. However, the anabolic condition of adipose tissue during early pregnancy switches to a net catabolic state as the gestation age progresses. The signals responsible for this switch are not well understood. As gestation age progresses maternal insulin sensitivity declines creating an insulin resistant state to meet the energy demands of both the mother and the rapidly growing foetus [43]. Maternal insulin resistance is reported to be mediated by increase in the levels of estrogen, progesterone, human placental lactogen, human placental growth hormone, cortisol, inflammatory cytokines [44, 45]. Elevated insulin resistance has been shown to be associated with development of dyslipidemia in the general population [46].

Hirschmugl *et al.*, suggested a relationship between PALP and maternal adiposity [47]. Hernández-Mosqueira *et al.*, suggested that the activity of tissue non-specific alkaline phosphatase (TNAP) might have a critical role in energy balance of the adipocyte, probably participating in obesity and metabolic syndrome [48]. Higher serum total ALP has been reported to be associated with incident [49] as well as prevalent metabolic syndrome [50]. Another study conducted by Krishnamurthy *et al.*, suggested that higher serum total ALP levels are strongly associated with increased prevalence of metabolic syndrome and subsequent increase in all-cause mortality in the US general population [51]. Their study recommended further determination of the causal molecular mechanism between the association of ALP and metabolic syndrome [51].

CONCLUSION

Associations between ALP and lipids have been reported in studies other than pregnancy. The present study shows that serum lipids increase in association with ALP during normal pregnancy. Since this study involved only healthy pregnant women, it cannot provide the patho-physiological mechanisms of the observed association; further studies are recommended in women with complicated pregnancies to gain insights into the patho-physiology of the association.

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