Original Article

Relationship of Serum Alkaline Phosphatase with Atherogenic Indices in Apparently Healthy Men, Makurdi, Nigeria

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Abstract

Background and Aim: Atherosclerotic calcification marked by elevated serum alkaline phosphatase (ALP) is linked to serum lipids. However, a normal lipid profile picture does not rule out ALP-associated pathogenesis. In a bid to better characterize the atherogenic potential of the routinely measured serum lipids, our study determined a relationship between ALP and atherogenic indices. **Methods:** Serum ALP, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin [ALB], lipids (total cholesterol, high-density lipoprotein-cholesterol [HDL-c], low-density lipoprotein-cholesterol [LDL-c], very LDL-c [VLDL-c], triglycerides [TGs], non-HDL-c [nHDL-c], remnant-cholesterol [remnant-c]), and atherogenic indices (Castelli's risk index [CAS-1 and CAS-2], atherogenic coefficient [AC], TG/HDL-c, atherogenic index of plasma [AIP]) were determined in 80 apparently healthy men aged 20–55 years. In addition, anthropometry, waist circumference, body mass index, and systolic and diastolic blood pressure (DBP) were determined. **Results:** According to ALP tertiles, a significant association ($P < 0.05$) of elevated ALP with increasing age, DBP, AST, ALT, VLDL-c, TGs, nHDL-c, remnant-c, CAS-1, CAS-2, AC, TG/HDL, AIP, and low HDL-c was observed in apparently healthy men. The study observed a significant negative correlation ($P < 0.02$) of ALP with HDL-c and ALB and positive correlation ($P < 0.05$) with AST, ALT, VLDL-c, TGs, nHDL-c, remnant-c, CAS-1, CAS-2, AC, TG/HDL, and AIP. **Conclusion:** The study has demonstrated that the atherogenic indices would be better markers than serum lipids in population studies of vascular calcification.

Keywords: Alkaline phosphatase, atherogenic indices, lipids

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Introduction

Atherosclerosis is a systemic vascular disease adjudged to be the underlying cause of cardiovascular diseases (CVDs), the major cause of death and morbidity in the industrial world.[1] Previous studies have observed hyperlipidemia as the major mechanistic risk factor of atherosclerotic vascular diseases, specifying an association between serum lipids and atherosclerotic calcification.[2] Vascular calcification is reported as part of the atherosclerotic plaque, with strong evidence suggesting that calcification volume measures adequately the total underlying plaque burden.[3] Early events of the vascular calcification process result from stimulated transdifferentiation of vascular smooth muscle cells (VSMCs) into alkaline phosphatase (ALP) secreting osteochondrogenic cells.[4]

ALP, usually measured in the evaluation of liver and bone disease, is shown to be an active promoter of vascular calcification in atherosclerosis and all-cause mortality.[5] ALP could be a marker of atherosclerosis since several clinical studies have shown its independent prediction of future cardiovascular events.[5,6] Stimulating factors of vascular calcification are under investigation.[7] There may be more than one mechanism since atherosclerosis itself occurs by several mechanisms.

It is well recognized that atherosclerosis is related to deposition and oxidation of lipoprotein components.[8] In an attempt to better characterize the atherogenic potential of serum lipids, mathematical derivatives of lipoproteins, Castelli's risk

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index (CAS-1 and CAS-2), atherogenic coefficient (AC),[9] triglyceride (TG)/high-density lipoprotein (HDL) ratio, atherogenic index of plasma (AIP).[10] Remnant-cholesterol (remnant-c), and non-HDL-cholesterol (nHDL-c) are determined in the recent studies as atherogenic markers in the prediction of CVDs.[10] Remnant-c, calculated as the cholesterol content of rich lipoproteins, is a strong causal risk factor for atherosclerotic CVD.[10] nHDL-c is determined as a valid surrogate to apolipoprotein B (apo B) 100 (apolipoprotein of low-density lipoprotein-cholesterol [LDL-c] and TG-rich lipoprotein) in the assessment of atherogenic cholesterol and lipoprotein burden.[11] It is established that the measurement of nHDL-c is a better predictor of CVD than LDL-c.[11] The use of nHDL-c (an equivalent of combined LDL-c and remnant-c) for CVD risk prediction has been emphasized in several guidelines and consensus papers.[10]

The emergence of a seeming contribution of lipids to atherosclerotic calcification stimulated our study, which aimed at investigating the association of ALP with atherogenic indices in apparently healthy men.

Materials and Methods

Participants and study design

We recruited male adults aged 20–55 years, attending a tertiary hospital in Makurdi for medical checkup. Participants with medical conditions were excluded from the study. Among the medical checkup attendees, 80 participants fulfilled the inclusion criteria and were included in the study. The study was approved by the institution's committee on research ethics and informed written consent was obtained from all study participants.

Anthropometric and biochemical measurements

All participants were required to fast for 12 h before intravenous blood sample collection for biochemical determinations. Physical examination was carried out by trained staff and physicians using the standard protocols.[12] Body weight and height were measured with the subject barefoot and wearing light clothing, and the values were used to calculate body mass index (BMI). Waist circumference (WC) was measured with the subject in the standing position and at the level of the umbilicus by a single examiner. Systolic and diastolic blood pressure (SBP and DBP) was measured twice in seated participants after a 5-min rest using a mercury sphygmomanometer. The mean values of these measurements were used in the analyses. Serum levels of total cholesterol (TC), TGs, HDL-c, albumin (ALB), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured adopting the colorimetric endpoint method using Randox reagent kits (Randox Laboratories Ltd., County Antrim, UK) on a spectrophotometer (Optima SP-300 Spectrophotometer; Optima INC. Tokyo, Japan). ALP obtained from Quimica Clinica Aplicada, S.A., Spain, was analyzed using the colorimetric endpoint method. Friedewald's equation was used in estimating very LDL-c (VLDL-c) and LDL-c.[13] nHDL-c was calculated as the difference between TC and HDL-c.[14] Remnant-c is estimated at TC minus LDL-c minus HDL-c, as done previously.[13] The atherogenic indices were calculated as follows; Castelli's risk index (CAS-1) = TC/HDL, CAS-2 = LDL/HDL, AC = (TC – HDL)/HDL, TG/HDL ratio,[9] and AIP = log (TG/HDL).[9]

Statistical analysis of data

Data were presented as means and standard deviations for continuous variables. To evaluate the factors associated with serum ALP level, the participants were grouped into tertiles based on serum ALP levels; first tertile (T1 < 53U/l), second tertile (T2 53–65U/l), and third tertile (T3 > 65U/l). 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 ALP and atherogenic indices as well as other variables. 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

The mean values of anthropometry (age, weight, WC, BMI, SBP, and DBP) and liver markers (ALB, AST, and ALT) according to ALP tertiles are shown in Table 1. The mean values of age, AST, and ALT in the first and second ALP tertiles differed significantly ($P < 0.01$) with the third tertile. Comparison of means of BP revealed that DBP in the first tertile differed significantly ($P < 0.05$) with the third tertile. The mean values of serum lipids (TC, HDL-c, LDL-c, VLDL-c, TGs, nHDL-c, remnant-c) and atherogenic indices (CAS-1, CAS-2, AC, TG/HDL, AIP) according to the ALP tertiles are shown in Table 2. The compared means revealed that HDL-c, VLDL-c, TGs, nHDL-c, remnant-c, CAS-1, CAS-2, AC, TG/HDL, and AIP in the third ALP tertile differed significantly ($P < 0.05$) with the first tertile. Apart from AI*P* value in the second tertile differing significantly ($P < 0.05$) with the first tertile, none of the lipids or atherogenic indices in the second tertile differed significantly ($P > 0.05$) with the first and third tertiles. Table 3 shows Pearson's correlation coefficients of ALP with anthropometry, liver markers, serum lipids, and atherogenic indices. ALP showed no correlation ($P > 0.05$) with anthropometry; correlated ($P < 0.05$) positively with VLDL-c ($r = 0.248$), TGs ($r = 0.225$), nHDL-c ($r = 0.252$), remnant-c ($r = 0.224$), AST ($r = 0.371$), ALT ($r = 0.335$), CAS-1 ($r = 0.330$), CAS-2 ($r = 0.271$), AC ($r = 0.330$), TG/HDL ($r = 0.246$), and AIP ($r = 0.291$); and correlated negatively with HDL-c ($r = -0.271$) and ALB ($r = -0.289$). However, ALB inversely correlated ($P < 0.05$) with TG/HDL ($r = -0.283$); remnant-c, CAS-1, AC, TG/HDL, and AIP correlated ($P < 0.01$) positively with AST ($r = 0.379, 0.333, 0.333, 0.550, 0.462$, respectively) and ALT ($r = 0.432, 0.253, 0.253, 0.549, 0.447$, respectively). Pearson's correlation coefficients between measured parameters in the study participants are presented in Table 4. Relevant to this study, results revealed that anthropometry did not correlate significantly ($P > 0.05$)

Table 1: Anthropometric parameters and liver markers according to alkaline phosphatase tertiles in adult men

Parameters	T1 (<53 U/l) (*n*=28)	T2 (53-65 U/l) (*n*=25)	T3 (>65 U/l) (*n*=27)	*F*	*P*
Age (years)	30.46±7.94	30.96±8.18	37.15±10.77	4.409	0.015*
Weight (kg)	65.57±9.85	65.76±7.68	67.26±10.09	0.266	0.767
WC (cm)	66.00±5.94	65.68±5.41	66.59±4.89	0.190	0.827
BMI (kg/m²)	23.09±2.94	23.16±2.40	23.77±3.16	0.464	0.630
SBP (mmHg)	121.89±6.72	123.52±4.61	123.30±7.13	0.533	0.589
DBP (mmHg)	78.96±7.63	80.72±6.55	82.70±6.26	2.046	0.136
ALB (g/l)	41.50±3.17	40.65±4.01	39.06±6.40	1.878	0.160
AST (U/l)	14.38±8.99	11.58±7.80	27.91±25.76	7.457	0.001*
ALT (U/l)	5.79±4.67	9.47±6.37	19.65±23.93	6.570	0.002*

Post hoc	T1	T2	*P*	T1	T3	*P*	T2	T3	*P*
Age	30.46±7.94	30.96±8.18	0.899	30.46±7.94	37.15±10.77	0.009*	30.96±8.18	37.15±10.77	0.016*
Weight	65.57±9.85	65.76±7.68	0.942	65.57±9.85	67.26±10.09	0.504	65.76±7.68	67.26±10.09	0.564
WC	66.00±5.94	65.68±5.41	0.831	66.00±5.94	66.59±4.89	0.688	65.68±5.41	66.59±4.89	0.547
BMI	23.09±2.94	23.16±2.40	0.933	23.09±2.94	23.77±3.16	0.381	23.16±2.40	23.77±3.16	0.441
SBP	121.89±6.72	123.52±4.61	0.350	121.89±6.72	123.30±7.13	0.411	123.52±4.61	123.30±7.13	0.898
DBP	78.96±7.63	80.72±6.55	0.355	78.96±7.63	82.70±6.26	0.047*	80.72±6.55	82.70±6.26	0.300
ALB	41.50±3.17	40.65±4.01	0.517	41.50±3.17	39.06±6.40	0.059	40.65±4.01	39.06±6.40	0.229
AST	14.38±8.99	11.58±7.80	0.538	14.38±8.99	27.91±25.76	0.003*	11.58±7.80	27.91±25.76	0.001*
ALT	5.79±4.67	9.47±6.37	0.363	5.79±4.67	19.65±23.93	0.001*	9.47±6.37	19.65±23.93	0.014*

*Significant at $P < 0.05$. WC: Waist circumference, BMI: Body mass index, ALB: Albumin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, SBP: Systolic blood pressure, DBP: Diastolic blood pressure

Table 2: Serum lipids and atherogenic indices according to alkaline phosphatase tertiles in adult men

Lipids (mmol/l) and atherogenic indices	T1 (<53 U/l) (*n*=28)	T2 (53-65 U/l) (*n*=25)	T3 (>65 U/l) (*n*=27)	*F*	*P*
TC	4.04±0.63	4.06±0.67	4.16±0.88	0.211	0.810
HDL-c	1.51±0.39	1.34±0.40	1.24±0.37	3.610	0.032*
LDL-c	2.15±0.59	2.27±0.76	2.40±0.84	0.828	0.441
VLDL-c	0.39±0.11	0.46±0.18	0.52±0.19	5.027	0.009*
TGs	0.84±0.25	1.02±0.39	1.12±0.43	4.278	0.017*
nHDL-c	2.53±0.63	2.72±0.62	2.93±0.82	2.231	0.114
Remnant	0.38±0.11	0.45±0.25	0.53±0.24	3.448	0.037*
CAS-1	2.84±0.90	3.29±1.01	3.61±1.10	4.046	0.021*
CAS-2	1.57±0.79	1.91±0.92	2.08±1.04	2.229	0.114
AC	1.84±0.90	2.29±1.01	2.61±1.10	4.046	0.021*
TG/HDL	0.60±0.28	0.86±0.50	1.08±0.97	3.758	0.028*
AIP	−0.26±0.19	−0.13±0.24	−0.05±0.24	6.472	0.003*

Post hoc	T1	T2	*P*	T1	T3	*P*	T2	T3	*P*
TC	4.04±0.63	4.06±0.67	0.933	4.04±0.63	4.16±0.88	0.546	4.06±0.67	4.16±0.88	0.615
HDL-c	1.51±0.39	1.34±0.40	0.099	1.51±0.39	1.24±0.37	0.010*	1.34±0.40	1.24±0.37	0.361
LDL-c	2.15±0.59	2.27±0.76	0.528	2.15±0.59	2.40±0.84	0.202	2.27±0.76	2.40±0.84	0.536
VLDL-c	0.39±0.11	0.46±0.18	0.080	0.39±0.11	0.52±0.19	0.002*	0.46±0.18	0.52±0.19	0.195
TGs	0.84±0.25	1.02±0.39	0.075	0.84±0.25	1.12±0.43	0.005*	1.02±0.39	1.12±0.43	0.312
nHDL-c	2.53±0.63	2.72±0.62	0.312	2.53±0.63	2.93±0.82	0.038*	2.72±0.62	2.93±0.82	0.300
Remnant	0.38±0.11	0.45±0.25	0.227	0.38±0.11	0.53±0.24	0.010*	0.45±0.25	0.53±0.24	0.183
CAS-1	2.84±0.90	3.29±1.01	0.113	2.84±0.90	3.61±1.10	0.006*	3.29±1.01	3.61±1.10	0.248
CAS-2	1.57±0.79	1.91±0.92	0.178	1.57±0.79	2.08±1.04	0.042*	1.91±0.92	2.08±1.04	0.507
AC	1.84±0.90	2.29±1.01	0.113	1.84±0.90	2.61±1.10	0.006*	2.29±1.01	2.61±1.10	0.248
TG/HDL	0.60±0.28	0.86±0.50	0.156	0.60±0.28	1.08±0.97	0.008*	0.86±0.50	1.08±0.97	0.219
AIP	−0.26±0.19	−0.13±0.24	0.031*	−0.26±0.19	−0.05±0.24	0.001*	−0.13±0.24	−0.05±0.24	0.206

*Significant at $P < 0.05$. TC: Total cholesterol, HDL-c: High-density lipoprotein-cholesterol, LDL-c: Low-density lipoprotein-cholesterol, VLDL-c: Very LDL-cholesterol, TG: Triglycerides, nHDL-c: NonHDL-cholesterol, CAS: Castelli's Risk Index, AC: Atherogenic coefficient, AIP: Atherogenic index of plasma

with liver markers, lipids, and atherogenic indices. However, TGs and VLDL-c correlated ($P < 0.01$) positively with AST ($r = 0.354, 0.346$) and ALT ($r = 0.404, 0.394$) but failed to correlate significantly ($P > 0.05$) with HDL-c and

Table 3: Pearson correlation coefficients of alkaline phosphatase with anthropometric parameters, serum lipids, liver markers and atherogenic indices

r	CAS 1	CAS 2	AC	TG: HDL	AIP	ALP
Age	0.054	0.042	0.054	0.027	0.07	0.139
BMI	0.182	0.203	0.182	0.033	0.104	−0.069
WC	0.121	0.11	0.121	0.055	0.101	−0.048
SBP	−0.144	−0.021	−0.144	−0.286*	−0.175	−0.018
DBP	−0.086	−0.023	−0.086	−0.141	−0.056	0.178
TC	0.183	0.333**	0.183	−0.271*	−0.119	0.096
HDL-c	−0.815**	−0.689**	−0.815**	−0.612**	−0.711**	−0.271*
LDL-c	0.579**	0.751**	0.579**	−0.133	0.091	0.181
VLDL-c	0.298**	0.07	0.298**	0.702**	0.797**	0.248*
TGs	0.294**	0.067	0.294**	0.707**	0.800**	0.225*
nonHDL	0.649**	0.732**	0.649**	0.067	0.279*	0.252*
Remnant	0.203	−0.098	0.203	0.668**	0.619**	0.224*
ALB	−0.177	−0.08	−0.177	−0.283*	−0.218	−0.289**
AST	0.333**	0.133	0.333**	0.550**	0.462**	0.371**
ALT	0.253*	0.038	0.253*	0.549**	0.447**	0.335**
CAS 1	1	0.923**	1.000**	0.567**	0.700**	0.330**
CAS 2		1	0.923**	0.224*	0.467**	0.271*
AC			1	0.567**	0.700**	0.330**
TG: HDL				1	0.866**	0.246*
AIP					1	0.291**
ALP						1

*Correlation is significant at the 0.05 level (2-tailed). WC: Waist circumference, BMI: Body mass index, ALB: Albumin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, TC: Total cholesterol, HDL-c: High-density lipoprotein-cholesterol, LDL-c: Low-density lipoprotein-cholesterol, VLDL-c: Very LDL-cholesterol, TG: Triglycerides, nHDL-c: NonHDL-cholesterol, CAS: Castelli's Risk Index, AC: Atherogenic coefficient, AIP: Atherogenic index of plasma

LDL-c. Serum ALB correlated positively ($P < 0.05$) with HDL-c ($r = 0.270$).

Discussion

The clinical importance of ALP in cardiovascular health is increasingly recognized. This association is supported by the experimental data which suggest that ALP activity is induced by stressors, with subsequent promotion of atherosclerotic calcification.[8] Circulating levels of ALP reflects ALP activity in tissues and may be a marker of atherosclerotic calcification in addition to liver and bone disease.[5] The role of serum lipids in the development of atherosclerosis is well established; however, its role in the promotion of vascular calcification is under investigation. This present study thus seeks to investigate the association of serum ALP levels with atherogenic indices that better characterize the atherogenicity of serum lipids. To eliminate the effect of confounding factors on ALP activity, our study participants were limited to 80 apparently healthy males.

According to ALP tertiles, we found an association of elevated ALP with increasing age, DBP, AST, ALT, VLDL-c, TGs, nHDL-c, remnant-c, CAS 1, CAS-2, AC, TG/HDL, AIP, and low HDL-c, in apparently healthy men. Despite unavailable data of an association of ALP with atherogenic indices, data closely related to the present study exist for an association of ALP percentiles with serum lipids, liver aminotransferases, BP, and age.[6,14,16,17] A prospective study by Wannamethee *et al*.[6] involving 3381 older British men, without CVD according to ALP quartiles, showed that ALP was strongly associated with old age and low HDL-c. Analysis of clinical characteristics of 1011 neurologically healthy participants by Lee *et al*.,[14] in a hospital-based cross-sectional study of ALP and cerebral small vessel disease, found a significant association of the third tertile of ALP with higher TGs levels compared with the first tertile. In a study of an association of ALP with metabolic syndrome involving 14,224 subjects, Kim *et al*.[16] found an association of increased quintiles of ALP with hypertriglyceridemia and high BP. After adjustment for age, sex, and BMI, higher ALP associated significantly with low HDL-c. A study by Krishnamurthy *et al*.,[17] involving 15,243 adults in the NHANES III database of associations of serum ALP with metabolic syndrome and mortality by serum ALP, showed that higher serum ALP levels were significantly associated with older age, higher AST, ALT, SBP, DBP, and TGs, and low HDL-c.

The present study observed a negative correlation of ALP with HDL-c and ALB and a positive correlation with AST, ALT, VLDL-c, TGs, nHDL-c, remnant-c, CAS 1, CAS 2, AC, TG/HDL, and AIP. This correlation seems to be independent of anthropometry since an absent correlation was observed between ALP and the anthropometric parameters. Likewise, our correlation results were similar to other previous studies,[16,18,19] except for atherogenic indices which had no available data. Kunutsor *et al*.,[18] using primary data from the prevention of

Table 4: Pearson correlation coefficients between measured parameters in the study participants

r	AGE	BMI	WC	TC	HDL-c	LDL-c	VLDL-c	TGs	nHDL	Remnant
Age	1									
BMI	0.199	1								
WC	0.396**	0.524**	1							
SBP	0.283*	−0.048	0.102							
DBP	0.362**	0.137	0.165							
TC	0.04	0.092	−0.038	1						
HDL-c	−0.025	−0.112	−0.1	0.323**	1					
LDL-c	0.032	0.155	−0.009	0.827**	−0.186	1				
VLDL-c	0.064	0.08	0.111	0.11	−0.213	−0.011	1			
TGs	0.041	0.057	0.07	0.126	−0.193	−0.005	0.984**	1		
nHDL	0.055	0.158	0.018	0.845**	−0.234*	0.955**	0.233*	0.238*	1	
Remnant	0.076	0.003	0.088	0.019	−0.149	−0.197	0.806**	0.801**	0.103	1
ALB	−0.082	0.044	0.063	0.161	0.270*	0.033	−0.083	−0.063	0.013	−0.069
AST	−0.066	0.034	−0.125	−0.122	−0.383**	−0.023	0.346**	0.354**	0.092	0.379**
ALT	−0.087	0.18	0.025	−0.137	−0.306**	−0.097	0.394**	0.404**	0.033	0.432**
CAS 1	0.054	0.182	0.121	0.183	−0.815**	0.579**	0.298**	0.294**	0.649**	0.203
CAS 2	0.042	0.203	0.11	0.333**	−0.689**	0.751**	0.07	0.067	0.732**	−0.098
AC	0.054	0.182	0.121	0.183	−0.815**	0.579**	0.298**	0.294**	0.649**	0.203
TG: HDL	0.027	0.033	0.055	−0.271*	−0.612**	−0.133	0.702**	0.707**	0.067	0.668**
AIP	0.07	0.104	0.101	−0.119	−0.711**	0.091	0.797**	0.800**	0.279*	0.619**

**Correlation is significant at the 0.01 level (2-tailed). WC: Waist circumference, BMI: Body mass index, ALB: Albumin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, TC: Total cholesterol, HDL-c: High-density lipoprotein-cholesterol, LDL-c: Low-density lipoprotein-cholesterol, VLDL-c: Very LDL-cholesterol, TG: Triglycerides, nHDL-c: Non-HDL-cholesterol, CAS: Castelli's Risk Index, AC: Atherogenic coefficient, AIP: Atherogenic index of plasma

renal and vascular endstage disease prospective study in the Netherlands, designed to investigate the association of ALP and CVD risk in a cohort of 6974 subjects, found a positive correlation of ALP with age, BMI, WC, SBP, dBP, ALT, TC, TGs and apo B and negative correlation with HDL-c. In the same study conducted by Kim *et al*.,[16] after correction for age, sex, and BMI, it was observed that ALP correlated positively with apo B and negatively with HDL-c level. Using the data of the open database of the United States NHANES 2005–2006, Webber *et al*.[19] analyzed the baseline characteristics of 4155 participants in the study for an association of ALP and C-reactive protein; in men, correlation of log ALP was significantly positive with age, WC, BMI, BP, ALT, and AST and negative with HDL-c and ALB. Without adjustment for gender, log ALP was positively correlated with age, WC, BMI, BP, AST, ALT, TC, TGs, and apo B and negatively with HDL and ALB.[19]

The relationship of serum lipids and atherogenic indices with ALP observed in the present study could be as a result of induction of ALP activity by lipids. The association of serum lipids with the atherosclerotic process has been documented.[2,8] This has informed the birth of the hypothesis "lipid-induced ALP activity." The expression of ALP gene, known as the tissue-nonspecific ALP (TNALP), is stimulated by transcription factors activated by certain inducers. TNALP is posttranslationally modified yet under the influence of unknown regulators to produce isozymes of TNALP; kidney, bone, liver, intestinal, placental, and germ-cell. TNALP is the most abundant ALP isozyme in the body, comprising >90% of circulating ALP. To support the existence of inducers of ALP activity, experimental studies in human osteoblasts and VSMCs suggested a direct association between the presence of mediators of inflammation in culture media and the induction of ALP activity in mineralizing cells.[20] Evidence also exists that oxidized lipids are inflammatory stimulus of vascular calcification that links hyperlipidemia with vascular calcification.[8] Vascular calcification has been shown to be associated with TNALP and bone alkaline phosphatase (BALP) activity level.[21,4] ALP when transported to the cell membrane become attached to the outer layer, where they act as ectoenzymes that inactivate mineralization inhibitors; inorganic pyrophosphate and osteopontin by dephosphorylation.[22] The importance of TNALP in inducing vascular wall calcification was convincingly shown in mice with genetically modified TNALP expression in vascular wall layers; TNALP overexpression in VSMCs and in endothelial cells both resulted in ectopic calcification.[21] Four bone-specific isoforms of TNALP (B/I, B1x, B1, and B2) are expressed in cells of mesenchymal origin such as skeletal cells (hypertrophic chondrocytes, odontoblasts, and osteoblasts) and stimulated vascular cells (myocytes, pericytes, or fibroblasts).[4] Vascular calcification is associated with osteochondrogenic transformation of vascular cells and elevated BALP activity level.[4] Although calcifying human VSMCs have been shown to express BALP, most studies in human and animal models do not specify the measured TNALP isoform, which does not exclude that other isoforms could be involved in vascular calcification. Experimental data suggest that the induction of

vascular ALP activity by oxidative stress promotes vascular calcification via osteoblastic differentiation of vascular cells.[23] There is *in vitro* evidence that lipids stimulate osteoblastic differentiation in the artery while depressing osteoblastic and stimulating osteoclastic differentiation in the bone.[23] Previous observational studies have demonstrated the relationship of coronary artery calcification with LDL and nHDL-c levels.[24,25] Osteoblastic differentiation and mineralization of VSMCs are enhanced by minimally oxidized LDL.[8] In this study, despite an observed nonrelationship of LDL-c with ALP, nHDL-c adjudged to be a surrogate of LDL-c and better predictor of CVD than LDL-c[11] showed a relationship with ALP. This implies that nHDL-c could stimulate vascular calcification through similar mechanisms as modified LDL-c. Accumulating evidence has suggested the protective role of HDL in CVD processes.[26] A diabetes heart study found a significant inverse association of HDL-c genetic risk score with coronary artery calcification.[26] Parhami *et al*.[27] demonstrated that human HDL-c inhibits the spontaneous osteogenic differentiation and mineralization of calcified vascular cells *in vitro*. Reports suggest that the protective effects of HDL-c may encompass its ability to inhibit cytokine-induced inflammatory responses since proinflammatory cytokines contribute to early atherogenesis.[28] The observed inverse relationship between HDL-c and ALP in this study could be an attempt by HDL-c to play a protective role through the inhibition of vascular calcification. A large study provides strong evidence for the causal role of TGs in atherosclerotic vascular disease.[29] However, there are controversies regarding hypertriglyceridemia be an independent risk factor for CVD, since elevated TG levels are often associated with decreased levels of HDL-c and increased levels of small-dense LDL particles.[30] To clear this doubt, findings from large studies indicate that elevated levels of TGs, TG-rich lipoproteins (such as VLDL-c), and their remnants, independent of other atherogenic lipids such as HDL-c and small-dense LDL, are associated with increased risk of CVD.[31] Among possible mechanisms for this association is stimulation of the production of proinflammatory cytokines, previously linked with enhancement of vascular calcification.[20] Thus, in this present study, the observed relationship between TGs and ALP may imply that vascular calcification could be promoted by TGs through inflammatory mechanisms independent of HDL-c and LDL-c. This independence is supported by the observation of absent correlation of TGs with HDL-c and LDL-c in this study and another recent finding.[31] The observation of a relationship between ALP and lipids in this study could be explained by the potential of lipids to activate ALP, orchestrated by osteochondrogenic transformation of vascular cells.

Since a number of routine lipid profile results appear nonatherogenic even in atherosclerotic settings, serum lipids have been used to calculate atherogenic indices (CAS-1, CAS-2, AC, TG/HDL, AIP), remnant-c, and nHDL-c to better characterize the atherogenic potential of lipid profile. Bendzala *et al*. recently observed that AIP which reflects the true relationship between protective and atherogenic lipoprotein was positively associated with the risk of all-cause death.[32] However, LDL-c, TC, TGs, and nHDL concentrations were not associated with the risk of death in these elderly women.[32] Nimmanapalli *et al*. assessed the cardiovascular risk of type 2 diabetics using lipid ratios; they found an association of CAS-1, CAS-2 with both complicated and noncomplicated type 2 diabetes mellitus patients.[33] Bhardwaj *et al*.[15] explained that AC reflects the atherogenic potential of the entire spectrum of lipoprotein fractions making it an indicator of CVD risks. da Luz *et al*.[9] showed that the ratio of TGs to HDL-c was found to be a powerful independent indicator of extensive coronary disease. Nordestgaard[10] reported that calculated remnant-c, which refers to the cholesterol content of all TG-rich lipoproteins, is a strong causal risk factor for atherosclerotic vascular disease. Several guidelines and consensus papers have emphasized the use of nHDL-c, a representative of LDL-c and remnant-c combination for CVD risk prediction.[10] The present findings of a relationship of all the atherogenic indices with serum ALP attest to their better characterization of atherogenicity than serum lipids. In addition, despite an absent relationship of ALP with TC and LDL-c in this study, a relationship of remnant-c and nHDL-c with ALP indicates atherogenicity in the face of normal TC and LDL-c.

The relationship of serum ALP with AST, ALT, and ALB (liver markers) observed in this study indicates the presence of an underlying subclinical liver dysfunction, probably contributing to circulating ALP levels in the study population. This is supported by a previous finding that found an association of liver enzymes with CVDs in the general population.[34] Our present findings of a positive correlation of TGs, TG-rich lipoproteins, atherogenic indices with AST and ALT; an inverse correlation of ALB with TG/HDL; and a positive correlation with HDL-c indicate that the subclinical liver dysfunction in the study population is of fatty origin. We suggest that the source of circulating ALP levels in the study participants is partly hepatic since nonalcoholic fatty liver is associated with isolated elevated ALP levels.[35] However, irrespective of the source of ALP, increased expression of ALP gene is associated with increased mortality and cardiovascular events via mechanisms that involve vascular calcification, endothelial dysfunction, and inflammation.[36] Furthermore, mechanisms related to subclinical liver dysfunction have been implicated in the association between ALP and CVDs.[5]

Peripheral vascular resistance to blood circulation due to vascular calcification could explain the association of elevated ALP with DBP observed in the present study. This is backed by reports of Perticone *et al*.,[37] who showed that serum ALP negatively affects endothelium-dependent vasodilation in naive hypertensive patients. The association observed between increasing age and ALP in this present study is backed by reports of defective mineralization in the aged.[38] Aging has been characterized with defects in physiological mineralization evidenced by osteoporosis and pathological mineralization, resulting in cardiovascular calcification with increased morbidity and mortality.[38]

Conclusion

To the best of our knowledge, from the literature search, a relationship between atherogenic indices and ALP activity has not been reported. However, few reports exist about an association between routinely measured serum lipids and ALP. Our study has demonstrated that the atherogenic indices would be better markers than serum lipids in population studies of vascular calcification, since ALP related with all the atherogenic indices unlike the serum lipids. Our observation of a correlation of TGs with ALP, but absent correlation of TGs with HDL-c and LDL-c, supports the independent role of TGs in mediating CVD as suggested by another recent study. Further studies involving a careful selection of a larger sample population size including females are recommended.

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Conflicts of interest

There are no conflicts of interest.

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