Clinica Chimica Acta 425 (2013) 192–195

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Association of alkaline phosphatase with acute myocardial infarction in a population with high prevalence of hypovitaminosis D

Mohammad Perwaiz Iqbal [a,*], Naseema Mehboobali [a], Iqbal Azam [b], Asal Khan Tareen [c]

[a] Department of Biological and Biomedical Sciences, Aga Khan University, Karachi 74800, Pakistan
[b] Community Health Sciences, Aga Khan University, Karachi 74800, Pakistan
[c] National Institute of Cardiovascular Diseases, Karachi, Pakistan

ARTICLE INFO

Article history:
Received 6 July 2012
Received in revised form 30 July 2013
Accepted 5 August 2013
Available online 15 August 2013

Keywords:
Acute myocardial infarction
Alkaline phosphatase
Bone alkaline phosphatase
Coronary heart disease
Hypovitaminosis D
Vitamin D deficiency

ABSTRACT

Background: Since Pakistanis have high prevalence of hypovitaminosis-D as well as acute myocardial infarction (AMI), the objective of the study was to investigate the relationship between vitamin-D deficiency and risk of AMI in a hospital-based population and to identify major risk factors for this disease.
Methods: Fasting serum samples from 66 consecutive AMI patients [age 30–70 y] and 132 gender and age-matched (within 5 y) healthy controls were analyzed for concentrations of glucose, total-cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, calcium, inorganic phosphate, alkaline phosphatase (ALP), bone-ALP, parathyroid hormone (PTH), 25(OH) vitamin-D (25(OH)D) and alanine aminotransferase.
Results: Mean concentrations of serum 25(OH)D, PTH, total-ALP, bone-ALP, LDL-cholesterol, HDL-cholesterol and glucose were significantly different compared to healthy controls ($p < 0.05$). Percent vitamin-D deficiency/insufficiency (levels < 30 ng/ml) was significantly greater in AMI patients compared to controls (93.9% vs.75.8%; $p = 0.001$). Multiple conditional logistic regression analysis revealed that increased levels of 25(OH)D were associated with decreased risk of AMI [MAOR (95% CI) = 0.821 (0.718, 0.940); $p = 0.004$]. Hypertension and smoking were positively associated with AMI.
Conclusions: Increased vitamin-D levels were associated with decreased risk of AMI, while serum glucose, bone-ALP, hypertension and smoking were positively associated with it. Association of bone-ALP with AMI in hypovitaminosis-D is a novel finding of this study.

1. Introduction

A number of studies have indicated an association between vitamin D deficiency and cardiovascular disease (CVD) [1–3]. Evidence towards lack of association between vitamin D deficiency and CVD has also been presented for certain populations or high risk individuals [4,5]. This indicates that hypovitaminosis D and the risk of developing CVD could vary from one population to another.

Pakistanis belong to a population group in which CVD risk factors and acute myocardial infarction (AMI) are on the rise [6]. It has also been reported that there is a high prevalence of vitamin D deficiency in this group [7,8]. While, quite a few studies have been carried out in the West to investigate the association of vitamin D deficiency with AMI, there are only few reports from South Asian region on it. The objective of the present study was to investigate the relationship between vitamin D levels and the risk of AMI in a hospital based population in Karachi, and to identify major risk factors in the development of this disease.

2. Subjects and methods

Sixty-six consecutive patients with AMI (age range30–70 y) admitted to the National Institute of Cardiovascular Diseases (NICVD), Karachi were enrolled in this study with informed consent. All these patients were Pakistanis and were identified with AMI for the first time. The diagnosis was based on WHO criteria of clinical history suggestive of myocardial ischemia, ECG indications of myocardial damage and elevated levels of biochemical markers — creatine kinase and troponin I. They were also assessed for other risk factors for coronary artery disease such as hypertension (systolic blood pressure > 140 mm Hg and diastolic blood pressure > 90 mm Hg), diabetes mellitus (fasting serum glucose > 125 mg/dl), abnormal lipid profile (total cholesterol > 200 mg/dl; low density lipoprotein (LDL)-cholesterol > 130 mg/dl; high density lipoprotein (HDL)-cholesterol < 40 mg/dl and triglycerides > 200 mg/dl). In addition, information about obesity (BMI > 27), smoking, parental history etc. was also taken into account. Exclusion criteria

Abbreviations: ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; AMI, Acute myocardial infarction; MAOR, Matched adjusted odds ratio; MI, Myocardial infarction; MOR, Matched odds ratio; NICVD, National Institute of Cardiovascular Diseases.

* Corresponding author at: Department of Biological & Biomedical Sciences, Aga Khan University, Stadium Road, P.O. BX-3500, Karachi-74800, Pakistan. Tel.: +92 21 3486 4463; fax: +92 21 3493 4294.
E-mail address: perwaiz.iqbal@aku.edu (M.P. Iqbal).

0009-8981/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.cca.2013.08.009

included pregnant females, patients with major illness such as malabsorption syndrome, cancer, liver disease and uremia and those taking vitamin D supplementation during the past 6 months. One hundred and thirty-two, apparently healthy subjects, who had been matched for age (within 5 y) and gender, were also recruited in the study with informed consent. They were the employees of the NICVD and the Aga Khan University and belonged to a similar socio-economic background and were included after clinical examination. They had no history of taking vitamin D supplements during the last 6 months and were not pregnant or suffering from malabsorption syndrome, cancer, liver disease, uremia, diabetes mellitus or coronary artery disease. Each AMI patient was matched for age (within 5 y) and gender with 2 controls. The study had been approved by the Ethics Review Committee of the Aga Khan University.

2.1. Determination of glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, calcium, phosphate, alkaline phosphatase, alanine aminotransferase, parathyroid hormone and 25(OH) vitamin D

Fasting venous blood was obtained within 24 h of AMI. Similarly, fasting blood was also obtained from healthy controls. Serum concentrations of glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, calcium, phosphate, alkaline phosphatase (ALP), alanine aminotransferase (ALT), parathyroid hormone (PTH) and 25(OH) vitamin D [25(OH)D] were determined using commercially available kit methods (Roche Diagnostic).

2.2. Measurement of bone alkaline phosphatase

Bone alkaline phosphatase (bone-ALP) in the serum was measured using a combination of a colorimetry-based kit (Roche) and heat inactivation method [9,10]. In this assay, each serum sample was divided into 3 parts A, B and C (200 μl each). Part A was subjected to colorimetric kit method for determination of total ALP in Cobas III analyzer following manufacturer's instructions. Part B was placed in a water-bath at 65 °C for 5 min and then immediately cooled in an ice bath. Thermal incubation at 65 °C leads to 100% inactivation of bone and liver isoenzymes. Part C was placed in a water bath at 55 °C for 16 min and immediately cooled in the ice bath. This incubation leads to 95% inactivation of bone-ALP and some liver-ALP [10]. ALP in sample part B and part C was measured using the colorimetry-based kit method. Total concentration of liver-ALP and bone-ALP was determined by subtracting the residual ALP activity in sample B from sample A, while concentration of bone-ALP was determined by subtracting the residual ALP activity in sample C (contributed mainly by liver-ALP) from sample A. The intra-assay CV of the bone-ALP was 8.8%, while inter-assay CV was 13.5% when the same samples were measured in assays run on different dates. The minimum limit of detection of this isoenzyme in the assay was 3 U/l.

2.3. Statistical analysis

Demographic and clinical quantitative values have been presented as mean ± S.E. Comparison of the mean values pertaining to AMI and two matched healthy controls was carried out using one-way ANOVA followed by Tukey's HSD pairwise comparisons test for significant mean differences. The association of vitamin D deficiency/insufficiency status (yes or no) and AMI status (yes or no) was observed using chi-square test. Multiple conditional logistic regression was used to investigate the relationship between AMI and vitamin D serum levels controlling for other risk factors including glucose, bone-ALP, high blood pressure and smoking. A $p < 0.05$ was considered significant.

2.4. Vitamin D insufficiency

Vitamin D insufficiency was defined as levels of 25(OH) D < 30 ng/ml [11].

3. Results

Demographic and clinical characteristics of both AMI patients and normal healthy subjects showed that mean concentrations of serum 25(OH)D, PTH, total ALP, bone-ALP, cholesterol, LDL-cholesterol, calcium, phosphate and glucose in AMI patients were significantly different compared to normal healthy controls ($p < 0.05$; Table 1). Percent vitamin D deficiency/insufficiency was significantly greater in AMI patients compared to age and gender-matched healthy controls (93.9% vs. 75.8%; $p = 0.001$; Table 2). Moreover, this deficiency/insufficiency was more pronounced in AMI male patients compared to healthy males (93.2% vs. 69.2%; $p = 0.001$; Table 2). Contributions of mean levels of bone-ALP to mean concentration of total ALP in both AMI patients and healthy controls were 83.4% and 83.2%, respectively; indicating little contribution by other isoenzymic forms of ALP.

Multiple conditional logistic regression analysis revealed that increased levels of 25(OH)D were associated with decreased risk of AMI [MAOR (95%CI) = 0.789 (0.722, 0.862); $p < 0.001$; Table 3]. Similarly, high levels of HDL-cholesterol were also found to be associated with decreased risk of AMI. On the other hand, serum concentrations of PTH, glucose, calcium, total ALP and bone-ALP, smoking and high blood pressure were found to be positively associated with the risk of AMI in the study population. When the model was adjusted for 25(OH)D levels, the pattern remained similar except for serum cholesterol, LDL-cholesterol and phosphate which became insignificant [Table 3].

Multivariable analysis also showed that increased levels of 25(OH)D were significantly associated with the decreased risk of AMI [MAOR (95%CI) = 0.821 (0.718, 0.94), $p = 0.004$; Table 4], controlling for glucose, bone-ALP, high blood pressure and smoking. However, smoking was the major risk factor for AMI in this population [MAOR (95%CI) = 22.63 (3.12, 164.22); $p = 0.002$].

Table 1
Demographic and clinical characteristics of patients with acute myocardial infarction and age and gender matched healthy subjects (controls). Mean ± SE.

Characteristic	AMI patients	Healthy controls		p-Value
	$n = 66$	Control 1 $n = 66$	Control 2 $n = 66$	
Age (y)	51.7 ± 1.04	50.26 ± 0.8	50.31 ± 0.8	NS
BMI [kg/m^2]	25.65 ± 0.37	24.65 ± 0.57	26.2 ± 0.65	NS
Glucose (mg/dl)	161 ± 9.3*[a]	102 ± 8.0	98.6 ± 5	<0.001
Cholesterol (mg/dl)	195 ± 6.2*[b]	173 ± 5	173 ± 6	0.007
Triglycerides (mg/dl)	177 ± 12.5	171 ± 14.0	158 ± 10	NS
HDL-cholesterol (mg/dl)	34.5 ± 1.2	37.5 ± 1.2	37.2 ± 1.2	NS
LDL-cholesterol (mg/dl)	125 ± 6*[c]	101 ± 4	102 ± 6	0.008
25(OH) vit D (ng/ml)	18.47 ± 0.66*[a]	26.5 ± 0.8	25.6 ± 1.0	<0.001
PTH (pg/ml)	65.6 ± 7.46*[a]	31.25 ± 3.4	32.25 ± 3.6	<0.001
Calcium (mg/dl)	9.04 ± 0.1*[d]	7.9 ± 0.2	8.5 ± 0.2	<0.001
Phosphate (mg/dl)	3.8 ± 0.11*[e]	3.4 ± 0.08	3.6 ± 0.07	0.002
Total ALP (U/l)	72.1 ± 3.1*[a]	56.0 ± 2.0	54.8 ± 1.7	<0.001
Bone-ALP (U/l)	58.1 ± 2.6*[a]	46.4 ± 1.9	45.0 ± 1.8	<0.001
ALT (U/l)	6.0 ± 2.1	6.3 ± 2.0	6.2 ± 2.0	NS

* p-Value < 0.05. Mean values in column related to AMI patients were compared with mean values in the corresponding columns related to 2 matched healthy controls using one-way ANOVA followed by Tukey's HSD pairwise comparisons.

[a] $p < .0001$ vs. controls 1 and 2.

[b] $p = 0.022$ and 0.013 vs. controls 1 and 2.

[c] $p = 0.015$ and 0.023 vs. controls 1 and 2.

[d] $p < 0.001$ and 0.047 vs. controls 1 and 2.

[e] $p = 0.001$ and NS vs. controls 1 and 2.