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Current Vascular Pharmacology, **2017,** *15,* **000-000** **1**

RESEARCH ARTICLE

Vitamin D is Related to Markers of Vulnerable Plaque in Acute Myocardial Infarction

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Abstract: ***Background:*** Vitamin D is a fat soluble vitamin involved in calcium and bone metabolism; recently its deficiency has been related to cardiovascular disease. In cardiac tissue, vitamin D suppresses metalloproteinases (MMPs) expression, enzymes directly associated with vulnerable plaque.

Objective: To investigate whether the association between vitamin D and leptin is related to markers of vulnerable plaque, such as MMPs in patients with acute myocardial infarction.

Methods: We studied 66 male patients with acute myocardial infarction, undergoing primary angioplasty. Blood samples were obtained at admission and 24hs after the surgery. Leptin and vitamin D concentrations in serum and MMP-2 and -9 activities in plasma were determined.

Results: MMP-2 activity was increased in Vitamin D deficient/insufficient patients at admission (p=0.04) and 24 hs later (p=0.05). In a linear regression model, vitamin D explained 24% of the variance of MMP-2 activity (F=2.839 p=0.04). At admission, vitamin D correlated with serum leptin (r=-0.302 p=0.033), and explained 39.5% of its variation (F=4.432 p=0.003).

Conclusions: In the studied population, vitamin D was inversely related to MMP-2 and leptin which are involved in coronary artery disease and acute myocardial infarction. The decrease in this hormone levels would be associated with a worse metabolic profile in acute coronary syndrome patients.

ARTICLE HISTORY

Received: April 06, 2017
Revised: May 19, 2017
Accepted: May 28, 2017

DOI:
10.2174/1570161115666170609102506

Keywords: Vitamin D, Acute myocardial infarction, Matrix metalloproteinases, Leptin, non HDL-cholesterol, Vulnerable plaque.

1. INTRODUCTION

Vitamin D is a fat soluble vitamin that plays an essential role in the calcium and bone metabolism [1]. In recent years, there has been an increased interest in other roles of the Vitamin D besides calcium metabolism. Different studies related vitamin D deficiency to insulin resistance [2], type 2 diabetes mellitus [3] and cardiovascular disease (CVD) [4].

In urban populations, it is reported that vitamin D levels are usually below the reference value of 30 ng/ml (74.9 nmol/l) [5]. Moreover, it has been found that severe deficiency of vitamin D (less than 10 ng/ml -25.0 nmol/l-) was an independent predictor of in-hospital cardiovascular mortality in patients with acute coronary syndrome [6]. Among the possible effects of vitamin D deficiency related to CVD, it has been reported that vitamin D suppress the renin-angiotensin system [7], affects endothelial function [8], upregulates thrombomodulin, and regulates macrophage activity [9].

Atherosclerotic plaque rupture is a major cause of acute coronary syndrome. Several mechanisms such as matrix degradation have been implicated in this process and metalloproteinases (MMPs) play an important role in plaque instability [10,11]. Different MMPs, have been identified in atherosclerotic plaques and in regions of foam cell accumulation and have been directly associated with plaque remodeling as well as plaque vulnerability [10,11] and with myocardial remodeling [12]. Moreover, it has been suspected that MMPs would be partly responsible for the pathogenesis of coronary

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artery disease (CAD). Furthermore, levels of MMP-2 were found to be independent predictors of survival in patients after a myocardial infarction [12]. Previous studies have shown that MMP-9 expression, secretion and activity are inhibited by vitamin D in tuberculosis infection [13]. In animal studies, it has been demonstrated that vitamin D plays an important role in suppressing MMP expression in cardiac tissue [14]. However, no data have been reported in coronary patients.

Obesity is a well-known independent risk factor for CVD. Adipose tissue produce several molecules, including adipocytokines such as leptin, strongly correlated with adiposity [15,16] however, the role of leptin in acute myocardial infarction is under investigation. An inverse relationship between leptin and vitamin D in obese patients was found [17], but there are no reports about this relation in patients with acute myocardial infarction. Given the proposed role of vitamin D deficiency in obesity and cardiovascular disease, our aim was to investigate whether the association between vitamin D and leptin is related to markers of vulnerable plaque, such as MMPs in patients with acute myocardial infarction.

2. MATERIALS AND METHODS

The study population was comprised of 66 consecutive male patients who were at the Argerich Hospital, Buenos Aires, with an ST-segment elevation acute myocardial infarction (STEMI), referred for primary angioplasty, within 24 hs of onset of symptoms. The STEMI was defined by prolonged chest pain and ST-segment elevation $\geq$ 1mm in at least two consecutive leads, or a new or presumably new left branch bundle block. Half of the studied population was recruited in spring/summer seasons, and the other half in autumn/winter seasons.

As previously published [18], full clinical details were recorded for all patients by pro-forma, including demographic data, smoking, coronary risk factors, previous clinical history and treatment as well as, educational level and marital status. Patients with cancer, stroke, and other severe inflammatory diseases and other pulmonary or hepatic diseases, thyroid disease, patients with Cushing's disease or syndrome, as well as chronic renal failure patients on hormonal treatment, were excluded from the subsequent analyses. We also excluded patients with cardiogenic shock at admission and other cardiovascular diseases. Previous myocardial infarction (MI) was established by evidence of previous hospital admission and a discharge diagnosis of MI [19]. All studies were performed in accordance with the Declaration of Helsinki, and all procedures were carried out with the adequate understanding and written informed consent obtained from all participants before coronary angiography. The protocol was approved by the Ethics Committee of the Argerich Hospital and the Faculty of Pharmacy and Biochemistry, University of Buenos Aires.

Coronary angiography and primary angioplasty were performed according to standard protocols and techniques. All the patients were referred to the catheterization laboratory, Hemodynamic Unit, within 30 minutes after arriving at the Emergency Department, where they received 100 mg of aspirin, 300 mg of clopidogrel and intravenous nitroglycerine.

Blood samples were obtained at admission and 24 hs after the routine cardiac protocol, between 8:00-9:00AM. Serum and plasma samples were separated by centrifugation at 1500 × g for 5 min and stored at -70°C. Triglycerides (TG), total cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, creatinine, leptin and vitamin D determinations were performed in serum; plasma samples were preserved for MMP-2 and MMP-9 activity determination.

Total cholesterol, TG, fasting glucose and creatinine were measured using commercial enzymatic kits (Roche Diagnostics, Mannheim, Germany) in a Cobas C-501 autoanalyzer, CVi<1.9%, CVe< 2.4%. Glomerular filtration rate was estimated using CKD-EPI formula described elsewhere, expressed as ml/min/1.73 m^2. A previous work had validated this formula for STEMI patients [20].

HDL-cholesterol and LDL-cholesterol were determined by standardized selective precipitation methods, using phosphotungstic acid/MgCl2 and polyvinyl sulfate as precipitating reagents, respectively, with CVi<2.0% and CVe<3.0%. No HDL-cholesterol (No HDL-chol) was calculated as the difference between total cholesterol and HDL-cholesterol.

The TG/HDL-cholesterol index was used as a surrogate marker of insulin resistance. Serum leptin was determined by RIA-I125 (Millipore Corporation, Billerica, MA USA). The CVi and CVe were less than 6.2% over the entire range of concentrations tested.

25 (OH) vitamin D was measured by chemioluminicent method with Advia Centaur (Siemens, USA) with CVi<5.3% and CVe<11.9%.

MMP-2 and MMP-9 activity was measured in plasma by gelatinolytic zymography as previously described [21]. MMP-9 (84 kd [active form]) and MMP-2 (67 kd [active form]) were identified by molecular weight. Conditioned media from the promyelocyte U-937 cell line was used as activity standard. Coefficients of variation were 4.8% (CVi) and 8.6% (CVe). Enzyme activity was detected as colorless bands quantified using Scion-Image J software (Scion Corporation, Frederick, MD), and relative activity was expressed as a ratio to the internal standard (Relative Units, RU).

2.1. Statistical Analysis

We first tested the distribution of variables using normality tests (kurtosis and skewness). Pearson and Spearman correlations were computed between independent and dependent variables and between potential confounders and dependent variables. To investigate the differences between groups, we used the t test for normal continuous variables and Mann-Whitney test for non-parametric ones. Pearson or Spearman's correlation controlling for different covariates and a linear regression analysis were performed, to test the relationship between vitamin D and MMPs, leptin and non HDL-cholesterol. We tested the unstandardized residuals for normality in order to perform the regression analysis. The SPSS 19.0 software package (Chicago, IL) was used for statistical analysis. A $p < 0.05$ was considered significant.

3. RESULTS

Table **1** shows the characteristics of the study population according to vitamin D levels. In the whole population, 14% had deficient concentration of vitamin D (less than 25.0 nmol/L), 71% had insufficient concentration (between 27.5 and 72.4 nmol/L) and 15% had sufficient concentration (between 74.9 and 249.6 nmol/L). Regarding metabolic profile no differences were observed neither in lipoprotein profile nor in glucose levels among groups. Nevertheless, in the population under study, vitamin D correlated with LDL-cholesterol (r=-0.284 p=0.04), non HDL-cholesterol (r=-0.298 p=0.032) and serum leptin (r=-0.302 p=0.033). Vitamin D deficient patients showed higher leptin levels (p=0.017) than sufficient patients (Table **2**). In a linear regression model, vitamin D explained 28% of the variation in non HDL-cholesterol (F=2.750 p=0.033) and 39.5% of the

Table 1. Baseline characteristics of the studied population, according to their vitamin D levels.

n=66	Deficiency (less than 25.0 nmol/L) (n=9; 14%)	Insufficiency (between 27.5 and 72.4 nmol/L) (n=47; 71%)	Sufficiency (between 74.9 and 249.6 nmol/L) (n=10; 15%)
Age (years)	53±13	59±12	57±5
Systolic blood pressure (mmHg)	132±22	131±25	128±12
Diastolic blood pressure (mmHg)	78±2	78±14	76±10
Heart rate (bpm)	75±13	82±16	75±10
BMI (kg/m^2)	29.4±3.5	27.1±3.0	27.2±4.3
Waist circumference (m)	1.99±0.06	2.00±0.04	1.98±0.03
Smokers (%)	87*	54	22
Former smokers (%)	13	25	44
Sedentary (%)	75	55	67
Diabetes Mellitus (%)	13	20	22
Normal Weight (%)	0	22	22
Overweight (%)	50	51	44
Obese (%)	50	27	34
Glomerular filtration rate ($ml/min/1.73m^2$)	86.69±19.86	79.99±15.63	82.04±10.86

BMI: Body Mass Index.

*χ^2 test. p=0.027.

Table 2. Laboratory variables of the studied population, according to their vitamin D levels.

n=66	Deficiency (less than 25.0 nmol/L) (n=9; 14%)	Insufficiency (between 27.5 and 72.4 nmol/L) (n=47; 71%)	Sufficiency (between 74.9 and 249.6 nmol/L) (n=10; 15%)
Triglycerides (mmol/L)	1.6±0.4	1.4±0.7	1.2±0.3
Total cholesterol (mmol/L)	5.8±1.6	5.2±1.1	5.2±0.8
HDL-c (mmol/L)	0.8±0.2	0.9±0.2	0.8±0.2
LDL-c (mmol/L)	4.3±0.7	3.9±1.2	3.5±0.5
Apolipoprotein A (g/L)	1.2±0.1	1.1±0.2	1.1±0.1
Apolipoprotein B (g/L)	1.0±0.2	1.1±0.3	1.0±0.2
Glucose (mmol/L)	7.6±1.6	9.2±2.2	8.0±1.3
TG/HDL-c	4.8±1.7	3.7±2.1	3.3±1.2
NonHDL-c (mmol/L)	5.4±1.2	4.5±1.1	4.3±0.9
Total cholesterol/HDL-c	8.4±2.3	6.5±1.9	6.5±1.9
25 (OH) Vitamin D (nmol/L)	15.6±4.7	53.4±12.3*	89.0±9.6**
Leptin (μg/L)	15.3±10.3	9.6±5.2	5.0±2.2***

*p<0.001 vs Deficiency, **p<0.001 vs Deficiency and Insufficiency, ***p=0.017 vs Sufficiency. ANOVA+Bonferroni test.

variation of serum leptin (F=4.432 p=0.003), after controlling for age, smoking status, estimated glomerular filtration rate and waist circumference. In this population, vitamin D was not related with waist circumference nor with body mass index (p=ns).

As expected, vitamin D was higher in those patients that were hospitalized in spring/summer compared to patients hospitalized in autumn/winter period (p=0.007), and was significantly lower in smoker patients than in non-smokers (45.17 (21.22) nmol/L vs 63.40 (21.22) nnmol/L, p=0.002).

Regarding MMPs activities, a tendency to higher MMP-2 activity was observed in deficient group patients (p=0.06) than in sufficient patients, without differences in MMP-9 activity. However, when considering deficient/insufficient patients together, MMP-2 activity was significantly higher in these patients compared to those with sufficient vitamin D concentration at admission (1.00±0.12 vs 0.89±0.07 RU, p=0.04) and 24 hs later (1.00±0.14 vs 0.88±0.09 RU, p=0.05) (Table **3A** and **B**, Fig. (**1A** and **B**)). Vitamin D correlated with MMP-2 activity at admission (r=-0.310 p=0.05), and 24 hs later (r=-0.310 p=0.05) (Fig. **2A** and **B**). In relation to the season in which patients were recruited and smoking status, there were no differences in MMP-2 activity (p=ns). In a linear regression model, vitamin D explained 24% of the variance of MMP-2 activity at admission (F=2.839 p=0.04), after controlling for age, smoking status and estimated glomerular filtration rate. No association was found between vitamin D and MMP-9.

4. DISCUSSION

The current study examined the relation between vitamin D and leptin with MMPs and lipoprotein profile in patients with acute myocardial infarction. In this population, MMP-2 activity was higher in patients with deficient/insufficient concentration of vitamin D compared to those patients with sufficient concentration. Moreover, after performing a linear regression analysis, vitamin D explained 24% of the variance of MMP-2 activity at admission, even after controlling for age, smoking status and estimated glomerular filtration rate. Vitamin D was also related with serum leptin and with an adverse lipoproteins profile at admission.

It has been reported that vitamin D deficiency is a worldwide concern [22]. Vitamin D is synthesized by the skin from 7-dehydrocholesterol during exposure to ultraviolet ray in sunlight [23]. Given that we recruited the studied patients in two different times of the year, as expected, vitamin D was higher in those patients that were hospitalized in spring/summer compared to patients hospitalized in autumn/winter. The percentage of patients with sufficient concentrations of vitamin D was in accordance with previous

Table 3. A. Plasma MMP-2 activity showed a tendency to be higher in those patients with deficient concentration of Vitamin D, at admission and 24 hour after the surgery.

	Deficiency		Insufficiency		Sufficiency	
	At Admission	After 24 hs	At Admission	After 24 hs	At Admission	After 24 hs
MMP-2 Activity (RU)	1.05±0.19	1.06±0.18	0.99±0.11	0.99±0.13	0.89±0.07*	0.88±0.09*

*p=0.06 vs Deficiency. ANOVA+Bonferroni test.

Table 3. B. When considering Deficient/Insufficient patients together, MMP-2 activity was higher than in patients with sufficient concentration of Vitamin D, at admission and 24 hours after the surgery.

	Deficiency/Insufficiency		Sufficiency	
	At Admission	After 24 hs	At Admission	After 24 hs
MMP-2 Activity (RU)	1.00±0.12	1.00±0.14	0.89±0.07*	0.88±0.09**

*p=0.04 vs Deficiency/Insufficiency at admission **p=0.05 vs Deficiency/Insufficiency after 24 hs.
Student t Test.

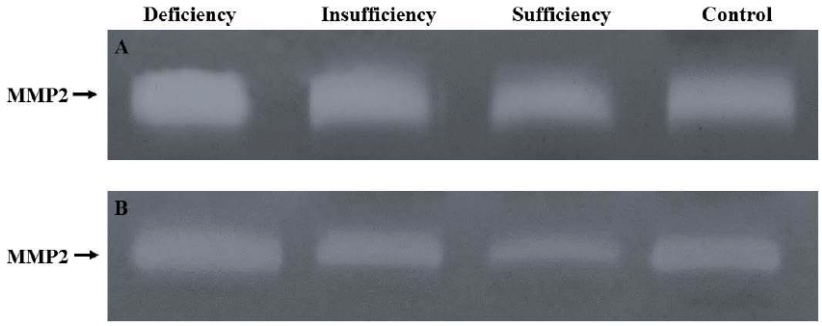

Fig. (1). Gelatinolytic Zymography of plasma MMP-2 activity of patients at admission (**A**) and 24 hours after the surgery (**B**).

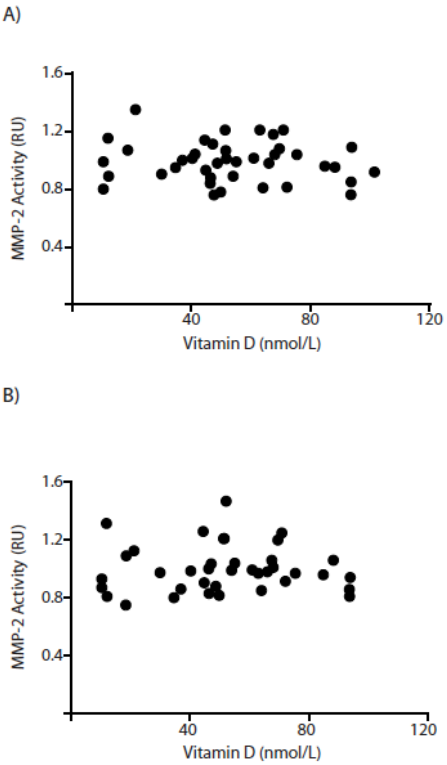

Fig. (2). Vitamin D correlated with MMP-2 activity at admission (r=-0.310, p=0.05) (**A**), and 24 hs later (r=-0.310 p=0.05) (**B**). Spearman test.

studies from others countries [5, 6] and with reported levels in hospitalized patients [24].

Traditional risk factors for cardiovascular disease have been studied in relation to vitamin D. Consistently with previous studies [25], our results indicate that smoker patients presented less vitamin D concentrations than non-smokers, independently of the season. The Very Large Database of Lipids (VLDL-3) study found that patients with vitamin D below 20 ng/ml (49.9 nmol/l) had lower HDL-cholesterol and higher LDL-cholesterol [26]. In our study, vitamin D was inversely correlated with LDL-cholesterol and non HDL-cholesterol, explaining 28% of the variation of non HDL-cholesterol.

Regarding the relationship between vitamin D and leptin, controversial results have been reported. Menendez *et al* described that vitamin D inhibit leptin secretion from human adipose tissue via a non-steroidal nuclear hormone receptor [27]. Moreover, only in obese patients, vitamin D was inversely related to serum leptin [23]. However, Breslavsky *et al* [28] showed that high doses of vitamin D supplementation did not change leptin levels in diabetic patients. In our study, vitamin D serum levels were inversely related to serum leptin, explaining 39.5% of the variation of serum leptin, independently of obesity. These results are in agreement with those reported by Khan *et al* [29] in 4000 healthy individuals, in which leptin is a mediator between vitamin D and different cardiovascular risk factors. Leptin has been proposed as a predictor of acute myocardial infarction, however more studies are necessary to clarify its role.

MMPs are implicated in the remodeling of extracellular matrix (ECM) during atherosclerosis [30]. Focal accumulation of cells that overexpress activated forms of MMPs may promote local destruction of ECM in atheroma, leading to plaque destabilization and rupture [30]. Furthermore, levels of MMP-2 were found to being dependent predictors of survival in patients after a myocardial infarction [12]. In this study, patients with deficient/insufficient concentration of vitamin D presented higher MMP-2 activity at admission and 24 hs later. Rahman A *et al* [14] showed that, in knockout mice for vitamin D receptor, MMP-2 and MMP-9 activities were higher in left ventricle than in wild type mice. It has been reported that vitamin D would impact on human and rat heart through a specific receptor [31]. In a prospective study, patients with acute ST segment elevation, vitamin D was inversely correlated with MMP-9 levels [32]. However, in our study, no relationship between MMP-9 activity and vitamin D was observed.

The present study included several limitations. First, the sample size; despite which, we conducted multiple statistical tests and several significant associations were observed. Secondly, only male patients were included into the study. Finally, our study lacked a normal control group in which it would be interesting to verify the present associations.

CONCLUSIONS

In the studied population, vitamin D was inversely related to MMP-2, leptin and non HDL-cholesterol, which are involved in coronary artery disease and acute myocardial infarction. The decrease in the levels of this hormone would be associated with a worse metabolic profile in acute coronary syndrome patients and further studies are necessary to validate these results.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Patients with acute myocardial infarction were recruited for this study. Refer to Materials and Methods.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author (editor) declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We thank the Agencia Nacional de Promoción Científica y Tecnológica and the Universidad de Buenos Aires for funding this research.

Funding: This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica (Grant number: PICT 2013-1150) and the Universidad de Buenos Aires (Grant number: UBACYT 20020150100042BA).

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