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Association of Serum Vitamin D, IL-4 Levels and Vitamin D Receptor Gene Polymorphism in Coronary Artery Disease with and Without Type 2 Diabetes Mellitus.

Ankit Gupta[1], Suresh Kumar[2]

[1]Senior Resident, Department of Medicine, Maulana Azad Medical College, New Delhi.
[2]Director Professor, Department of Medicine, Maulana Azad Medical College, New Delhi.

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ABSTRACT

Background: The aim of this study was to investigate the association of serum vitamin D, IL-4 levels and vitamin D receptor gene polymorphism in coronary artery disease with and without type 2 diabetes mellitus. **Methods:** The study was conducted in Department of Medicine and Department of Biochemistry, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi. It involved two groups of patients suffering from CAD with type 2 diabetes mellitus (n =40) and CAD without type 2 diabetes mellitus (n =40). Blood sample was collected from all subjects using all aseptic precautions. The levels of serum 25-hydroxy Vitamin D were measured by Electrochemiluminescence Immunoassay. Expected normal serum values considered was 14-80ng/ml. Serum IL-4 had been measured by using commercially available ELISA kit provided by GEN- PROBE Diaclone, France. Expected normal serum value considered was < 98pg/ml. **Results:** The mean age of patients in different study groups were CAD with DM, 59.15± 9.31 years and CAD without DM, 58.1±9.51 years. Mean vitamin D levels were 18.6±8.3 ng/ml in CAD with DM and 23.4±9 ng/ml in CAD without DM. Mean IL-4 levels were 1.31±0.27pg/ml in CAD with DM group, 1.21±0.29pg/ml in CAD without DM group. The FF genotype of vitamin D receptor gene was present in 47.5 % of CAD with DM patients and 35 % of CAD without DM patients. The Ff genotype was present in 37.5 % of CAD with DM patients and 52.5 % of CAD without DM patients. The ff genotype was reported in 15 % of CAD with DM patients and 12.5 % of CAD without DM patients. Allele F of Vitamin D receptor gene constituted 66 % of total gene pool in CAD with DM patients and 61 % in CAD without DM patients. No significant association was observed with respect to the VDR FokI genotypes and cardiovascular outcomes. **Conclusion:** Serum Vitamin D levels were decreased in both groups of patients, more significantly decreased in the presence of DM in CAD patients. Serum IL-4 levels were significantly higher in CAD with DM group as compared to CAD without DM group. No associations could be found between Vitamin D receptor gene FokI polymorphism and risk of CAD in diabetic and non-diabetic individuals. No significant correlation was found between vitamin D and IL-4 levels in the patients of both groups. The association between VDR FokI polymorphism, vitamin D and inflammatory markers needs to be further explored in diabetic CAD patients.

Keywords: Vitamin D, IL-4 Levels, Vitamin D Receptor Gene.

INTRODUCTION

Coronary artery disease (CAD) is a leading cause of death in developed countries and is rapidly assuming epidemic proportions in developing countries as well.[1] The risk factors associated with CAD include the non-modifiable risk factors such as sex, advancing age and family history and modifiable risk factors such as elevated cholesterol, hypertension, obesity and diabetes.[2] Even maximum modification of the above risk factors has not reduced the high incidence/severity of CAD. This is referred to as the famous INDIAN PARADOX.[3] Hence there is need to assess newer risk factors which could possibly explain this paradox. The root cause of the entire spectrum of coronary artery disease- angina, myocardial infarction, and stroke – lies in atherosclerosis.[4]

Atherosclerosis is the combination of two distinct processes – gradual deposition of lipids in arterial media and coronary thrombosis.[4] A new frontier of research is now focused on identifying these novel risk factors which promote and accelerate the atherosclerotic process and may account for the high incidence of CAD amongst Indians.[5] Recent epidemiologic and experimental evidence has suggested that low vitamin D levels may play a role in various cardiovascular conditions, including coronary artery disease, congestive heart failure, valvular calcification, stroke, hypertension, and cognitive decline. It had been shown that lower

Name & Address of Corresponding Author
Dr. Ankit Gupta,
Senior Resident,
Department of Medicine,
Maulana Azad Medical College,
New Delhi.

vitamin D levels appear to predict an increased risk of coronary artery mortality in patients with type 2 diabetes.[6,7]

Interleukin-4 (IL-4) was traditionally considered as an anti-inflammatory cytokine.[8] Recent studies have provided robust evidence that IL-4 exerts pro-inflammatory effects on vascular endothelium and may play a critical role in the development of atherosclerosis.[8] Vitamin D mediates its action in the body through Vitamin D receptor (VDR), a member of nuclear hormone receptor super family that modulates the transcription of target genes which help in calcium uptake or bone formation like calcium binding proteins and osteocalcin.[8] The gene encoding the VDR is located on chromosome 12cen-q12, contains 11 exons, and spans approximately 75 kilobases of genomic DNA. Allelic variants of the gene encoding VDR are recognized by ApaI (allele A/a), BsmI (allele B/b), FokI (allele F/f) and TaqI (allele T/t) restriction endonucleases.[9] The FokI polymorphism is a T→C transition polymorphism (ATG to ACG) at the first of two potential translation initiation sites in exon II has been defined using the FokI restriction endonuclease.[9]

In ff variant of FokI polymorphism of VDR gene, initiation of translation occurs at the first ATG site, giving rise to a full length VDR protein comprised of 427 amino acids.[10] Conversely, in the VDR FF variant, translation begins at the second ATG site instead of the first, resulting in a truncated protein with three amino acids lesser. The association of VDR polymorphisms in immunological disorders such as hepatitis B,asthma, multiple sclerosis, and type 1 diabetes are well established.[11-13] Recent studies have shown that the VDR gene is a novel candidate gene contributing to susceptibility to type 2 diabetes mellitus.[14,15] Therefore, the aim of this study was to investigate the association of serum vitamin D, IL-4 levels and vitamin D receptor gene polymorphism in coronary artery disease with and without type 2 diabetes mellitus.

MATERIALS AND METHODS

Aims & objectives

- To analyze the association of serum Vitamin D levels and coronary artery disease with Type 2 diabetes mellitus.
- To determine the association of Vitamin D receptor gene polymorphism and coronary artery disease with Type 2 diabetes mellitus.
- To find the association of serum IL- 4 levels in coronary artery disease with Type 2 diabetes mellitus.

Study site:

The study was conducted in Department of Medicine and Department of Biochemistry, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi.

Study design:

An observational study for the evaluation of association of serum vitamin D, IL-4 levels, vitamin D receptor gene polymorphism (rs2228570) in CAD with and without type 2 diabetes mellitus.

Study population:

The study involves two groups of patients suffering from CAD with type 2 diabetes mellitus (n =40) and CAD without type 2 diabetes mellitus (n =40) attending emergency or coronary care unit of Lok Nayak Hospital.

Inclusion criteria

- Patients with h/o acute coronary syndrome with and without type 2 diabetes mellitus
- Age > 45 yrs

Exclusion criteria

- Type 1 diabetes mellitus
- Chronic kidney disease
- Hyperparathyroidism, hypoparathyroidism
- Patients taking vitamin D or calcium supplements
- Patient with diagnosed malignancy

Study process

The present study was carried out after obtaining prior approval from the institutional ethical committee. Patients of CAD with type 2 DM (n=40) and CAD without type 2 DM (n=40) attending the emergency or coronary care unit of department of medicine, LNH, Delhi were enrolled for the present study after taking informed consent. Selection of patients was done by taking history of acute coronary syndrome or severe chronic CAD. A major symptom of coronary artery disease is retrosternal chest pain, characterized by a squeezing or tight sensation and that is usually associated with dyspnoea.

Acute coronary syndrome (ACS) refers to a spectrum of clinical presentations ranging from those for ST-segment elevation myocardial infarction (STEMI) to presentations found in non–ST-segment elevation myocardial infarction (NSTEMI) or in unstable angina. Symptoms of severe chronic stable angina are marked limitation of physical activity or inability to carry out any physical activity. The patient may have fatigue, anginal pain, dyspnoea or palpitations. Screening of patients for diabetes was done as per American Diabetes Association guidelines.

Diagnosis of diabetes mellitus

1. Classic symptoms of diabetes mellitus (polyuria, polydipsia, polyphagia and weight loss and random plasma glucose > 200 mg/dL
2. Fasting plasma glucose > 126 mg/dL
3. Two hour post glucose load (75g) plasma glucose > 200 mg/dL, and confirmed by repeat test

Patients had undergone a physical examination including general physical examination and examination of the cardiovascular system.

BMI Estimation

BMI = weight/height^2, with weight being in kilograms and height being in meters.

According to World Health Organization (WHO) classification:

- Grade 1 overweight (overweight) - BMI of 25-29.9 kg/m2
- Grade 2 overweight (obesity) - BMI of 30-39.9 kg/m2
- Grade 3 overweight (severe or morbid obesity) - BMI greater than or equal to 40 kg/m2

Patients with clinical features of acute coronary syndrome had undergone ECG and cardiac enzyme assay for diagnosis. Patients with clinical features of chronic CAD had undergone resting ECG. Both categories of patients had also undergone echocardiogram to detect regional wall motion abnormalities if required.

Sample collection

A total of 8ml of blood sample was collected from all subjects using all aseptic precautions. Three ml. of the sample was used for biochemical investigations. Two ml. of sample was taken in standard EDTA vials and stored at -80^o C. Detection of Vitamin D receptor gene polymorphism was done by using PCR-RFLP. Three ml. of sample was preserved in plain vials for quantification of serum Vitamin D by chemiluminescence immunoassay technique and IL-4 by ELISA.

Estimation of serum 25-hydroxy Vitamin D levels

The levels of serum 25-hydroxy Vitamin D were measured by Electrochemiluminescence Immunoassay. (ECL). Expected normal serum values considered was 14-80ng/ml.

Estimation of serum IL-4 levels

Serum IL-4 had been measured by using commercially available ELISA kit provided by GEN- PROBE Diaclone, France. Expected normal serum value considered was < 98pg/ml.

Detection of Vitamin D receptor gene foki (exon2 start codon) polymorphism (rs2228570)

Whole blood samples from patients were collected in EDTA vials for the study. The sample was stored in -80^{0}C till analysis. Analysis was done using Polymerase Chain Reaction (PCR).

The required region of vitamin D receptor gene from the genomic DNA was amplified by polymerase chain reaction using primers.

Statistical analysis

Observational data is being presented as mean and standard deviation or percentage. Parametric nature of data was verified by Kolmogorov – Smirnov test. For comparison of data between two groups, student t test or Mann - Whitney U test was used in parametric and non- parametric data respectively. Odds ratio associated with vitamin D receptor gene polymorphism in CAD with type 2 DM was calculated and 95% confidence interval was determined. For correlation analysis between vitamin D and IL-4 level, Spearman or Pearson's analysis was done after assessing the normal distribution of data. $P < 0.05$ is being considered as significant in all statistic tests.

RESULTS

Baseline characteristics

The present study comprised of 80 subjects of whom 40 patients had CAD with DM and 40 had CAD without DM. The mean age of patients in different study groups were CAD with DM, 59.15± 9.31 years and CAD without DM, 58.1±9.51 years (Table 1). A total of 65% of CAD with DM and 67.5% of CAD without DM were in the age group 45-60 yrs. Among the CAD with DM group, 65% patients were males and 35% were females while among the CAD without DM group, 75% were males and 25% were females. Mean BMI was significantly higher in CAD with DM (p=0.039) as compared to the patients of CAD without DM [Table 1].

Table 1: Baseline characteristics of study population

Age Interval (years)	CAD with DM(n=40)		CAD without DM(n=40)	
	N	%	N	%
45-60	26	65	27	67.5
61-75	12	30	11	27.5
76-90	2	5	2	5
Mean (SD)	59.15 (9.31)		58.1 (9.51)	
Gender				
Male	26	65	30	75
Female	14	35	10	25
Mean BMI*	26.2± 4.5		24.6± 3.5	

*Difference in mean BMI significant (P=0.039)

Table 2: Mean serum Vitamin D and IL-4 levels in CAD with DM and CAD without DM

GROUP	Vitamin D levels (ng/ml)		IL-4 levels (pg/ml)	
	Mean	S.D	Mean	S.D
CAD with DM (n=40)	18.6	8.3	1.31	0.27
CAD without DM (n=40)	23.4	9	1.21	0.29
p-value	0.007		0.047	

Biochemical Parameters

The serum levels of biochemical parameters – Vitamin D levels and IL-4 levels were compared between the CAD with DM and CAD without DM. As revealed by Electrochemiluminescence Immunoassay, mean vitamin D levels were 18.6±8.3 ng/ml in CAD with DM and 23.4±9 ng/ml in CAD without DM [Table 2]. The difference among these two groups was statistically significant (p=0.007). As revealed by ELISA, mean IL-4 levels were 1.31±0.27pg/ml in CAD with DM group,

1.21±0.29pg/ml in CAD without DM group. The difference among these two groups was statistically significant (p=0.047) [Table 2]. No significant correlation was found between vitamin D and IL-4 levels in the patients with CAD with DM (r=-0.1378; P=0.197). No significant correlation was found between vitamin D and IL-4 levels in the patients with CAD without DM (r=0.077; P=0.316).

Table 3: Vitamin D receptor FokI genotypes in CAD with DM and CAD without DM

	Groups		p value
	CAD with DM(n=40)	CAD without DM (n=40)	CAD with DM vs. CAD without DM(OR)
FF	19(47.5%)	14(35%)	0.12 (1.68)
Ff	15(37.5%)	21(52.5%)	0.08 (0.54)
Ff	6(15%)	5(12.5%)	0.50 (1.23)
F	53 (66%)	49 (61%)	0.25 (1.24)
F	27 (34%)	31 (39%)	0.25 (0.8)

OR=Odds Ratio

Analysis of vitamin D receptor gene FokI polymorphism

The Vitamin D receptor gene polymorphism was studied using FokI restriction enzyme. The FF genotype of vitamin D receptor gene was present in 47.5 %(n=19) of CAD with DM patients, 35 %(n=14) of CAD without DM patients (Table 3). The difference between CAD with DM vs. CAD without DM was not statistically significant (OR-1.68, 95% CI: 0.68-4.1). The Ff genotype was present in 37.5 %(n=15) of CAD with DM patients, 52.5 %(n=21) of CAD without DM patients (Table 3). The difference between CAD with DM vs. CAD without DM was not statistically significant. (OR-0.54, 95% CI: 0.22-1.3). The ff genotype was reported in 15 % (n=6) of CAD with DM patients, 12.5 %(n=5) of CAD without DM patients [Table 3]. The difference between CAD with DM vs. CAD without DM was not statistically significant. (OR-1.2, 95% CI: 0.34-4.4). Allele F of Vitamin D receptor gene constituted 66 %(n=53) of total gene pool in CAD with DM patients, 61 %(n=49) in CAD without DM patients (Table 3). The difference was found to be not significant for CAD with DM group as compared to CAD without DM. (OR-1.24, 95% CI: 0.65- 2.3). Allele f of Vitamin D receptor gene constituted 34 %(n=27) of total gene pool in CAD with DM patients, 39 % (n=31) in CAD without DM patients. The difference was found to be not significant for CAD with DM group as compared to CAD without DM. (OR-0.80, 95% CI: 0.42- 1.5) [Table 3].

Association of FokI genotypes with 25 hydroxyvitamin D levels and serum IL-4 levels in CAD with and without DM

25-hydroxyvitamin D levels were analyzed for 40 patients of CAD with DM, who were segregated into low vitamin D (<14ng/ml) and those falling under the normal range (14-80ng/ml). Out of 40 patients, 14 had low vitamin D and the remaining 26 were within the normal range. No significant association was observed with respect to the VDR FokI genotypes. (p=0.18) [Table 4]. IL-4 levels were analyzed for 40 patients of CAD with DM, who were segregated into normal IL-4 (< 98pg/ml) and those falling under the high range (>98pg/ml). Out of 40 patients, 11 had normal serum IL-4 levels and the remaining 29 were in the high range. No significant association was observed with respect to the VDR FokI genotypes. (p=0.29) [Table 4].

25-hydroxyvitamin D levels were analyzed for 40 patients of CAD without DM, who were segregated into low vitamin D (<14ng/ml) and those falling under the normal range (14-80ng/ml). Out of 40 patients, 9 had low vitamin D and the remaining 31 were within the normal range. No significant association was observed with respect to the VDR FokI genotypes (p=0.24) [Table 5]. IL-4 levels were analyzed for 40 patients of CAD without DM, who were segregated into normal IL-4 (<98pg/ml) and those falling under the high range (>98pg/ml). Out of 40 patients, 17 had normal serum IL-4 levels and the remaining 23 were in the high range. No significant association was observed with respect to the VDR FokI genotypes. (p=0.24) [Table 5].

Table 4: Association of FokI genotypes with 25 hydroxyvitamin D levels and serum IL-4 levels in CAD with DM

Genotypes	Low vitamin D (< 14ng/ml) % (n)	Normal vitamin D (14-80ng/ml) % (n)	p value (OR)	Normal serum IL-4 (< 0.98pg/ml) % (n)	High serum IL-4 (>0.98pg/ml) % (n)	p value (OR)
FF	42.1%(8)	57.9% (11)	0.18 (1.8)	31.6%(6)	68.4%(13)	0.29 (1.47)
Ff	26.6%(4)	73.3%(11)	0.19 (0.54)	20%(3)	80%(12)	0.20 (0.53)
Ff	33.3%(2)	66.6%(4)	0.46 (0.91)	33.3%(2)	66.7%(4)	0.36 (1.38)

Table 5: Association of FokI genotypes with 25 hydroxyvitamin D levels and serum IL-4 levels in CAD without DM

Genotypes	Low vitamin D (< 14ng/ml) % (n)	Normal vitamin D (14-80ng/ml) % (n)	p value (OR)	Normal IL-4 (< 0.98pg/ml) % (n)	High IL-4 (>0.98pg/ml) % (n)	p value (OR)
FF	28.6%(4)	71.4%(10)	0.24 (1.68)	50%(7)	50%(7)	0.24 (1.6)
Ff	14.3%(3)	85.7%(18)	0.09 (0.36)	42.8%(9)	57.1%(12)	0.48 (1.03)
ff	40%(2)	60%(3)	0.15 (2.66)	20%(1)	80%(4)	0.13 (0.29)

DISCUSSION

The present study was designed to analyze the association of serum vitamin D, IL-4 levels and vitamin D receptor gene polymorphism in coronary artery disease with and without type 2 diabetes mellitus. The study involved two groups of patients suffering from CAD with type 2 diabetes mellitus (n =40) and CAD without type 2 diabetes mellitus (n =40) attending emergency or coronary care unit of Lok Nayak Hospital. The mean age of study population was CAD with DM, 59.1±9.3years and CAD without DM, 58.1±9.5years. There was male predominance in both groups of patients. Mean BMI was significantly higher in CAD with DM as compared to the patients of CAD without DM. (p=0.039).

In the present study, serum vitamin D levels were found to be decreased in both CAD with DM and CAD without DM. The mean vitamin D levels were 18.6±8.3 ng/ml in CAD with DM and 23.4±9 ng/ml in CAD without DM. This suggests a strong association between vitamin D deficiency and CAD. A significant decrease in vitamin D levels was seen in CAD with DM as compared to CAD without DM patients (p=0.007). Association of Vitamin D status and coronary arterial disease (CAD) has been examined in both cross-sectional and prospective studies. A cross-sectional study of the NHANES III population revealed an association between vitamin D deficiency (25(OH) D <20 ng/ml) and CAD, defined as self-reported angina, myocardial infarction or stroke with an odds ratio of 1.20.[16] Similar results were seen in the Health Professionals Follow Up Study in which the RR for nonfatal MI or fatal cardiovascular disease was 2.42 in those with vitamin D deficiency.[17] In the Framingham Offspring Study the rate of cardiovascular events was also higher in those patients with 25(OH)D levels <15 ng/ml.[18] Randomized clinical trials designed to assess CAD as a primary outcome are not available; however, several studies have examined this relationship in secondary analyses.[19,20] Overall, results fail to demonstrate a significant effect of vitamin D supplementation with or without calcium, but a trend towards benefit was seen.[19,20]

In a recent randomized trial in New Zealand,[21] vitamin D supplementation was found to improve insulin resistance. This study primarily involved women of South Asian descent who were randomized to receive vitamin D supplementation (4000 IU per daily) or placebo for 6 months. Insulin resistance was assessed by homeostatic model assessment (HOMA). A significant improvement in insulin sensitivity and fasting insulin levels were observed. Of note, improvement was not seen before 6 months of therapy and not until vitamin D reached levels >80 nmol/L. In addition, a study of 459 type 2 diabetic patients showed a strong inverse association between 25(OH)D3 levels and prevalent CVD.[22] In order to provide more robust evidences on vitamin D in prevention and management of type 2 diabetes, future trials should also investigate the role of higher doses of vitamin D supplementation in larger populations and for longer periods.

As revealed by our study, serum IL-4 levels were statistically increased in CAD with DM group as compared to CAD without DM group(p=0.047). Mean IL-4 levels were 1.31±0.27pg/ml in CAD with DM group, 1.21±0.29pg/ml in CAD without DM group. Serum IL-4 levels were increased in both groups of patients. There is paucity of literature regarding the association of IL-4 in diabetic CAD in the Indian setup. Recent studies have provided evidence that IL-4 play a critical role in the development of atherosclerosis. Huang et al.[23] had shown that IL-4 induces pro-inflammatory environments by over expressing a number of inflammatory mediators in vascular endothelial cells. In a study by Blease et al. it was observed that IL-4 synergistically increases interleukin-1β (IL-1β)-, tumor necrosis factor-α (TNF-α)-, or lipopolysaccharide (LPS)-induced vascular cell adhesion molecule-1 (VCAM-1) expression in vascular endothelium.[24] Lee et al. had demonstrated that IL-4 increases endothelial cell turnover by accelerated apoptosis, the event which may alter the function of the vascular endothelium and thereby promote atherogenesis.[25] They also provided robust evidence that intracellular ROS generation is associated with IL-4-induced vascular inflammation.[25] King et al. demonstrated that transplantation of bone marrow stem cells collected from IL-4-deficient mice led to decreased atherosclerotic lesion formation in LDLR-deficient mice.[26]

Vitamin D is an important dietary factor that mediates its action in the body through Vitamin D receptor (VDR).[4] VDR gene has also been suggested as one of the candidate genes for genetic control of bone mass. Allelic variants of the gene encoding VDR recognized by ApaI (allele A/a), BsmI (allele B/b), FokI (allele F/f) and TaqI (allele T/t) restriction endonucleases.[9-11] The FokI polymorphism is a T→C transition polymorphism (ATG to ACG) at the first of two potential translation initiation sites in exon II has been defined using the FokI restriction endonuclease.[9,10] The TaqI polymorphism is a T→C nucleotide substitution (ATT to ATC) leading to a synonymous change at codon 352 (isoleucine) in exon IX. Bsm I and ApaI restriction site polymorphisms occur in the intron separating exons VIII and IX.[12,13] VDR is involved in vascular smooth muscle cell growth and in the regulation of calcium homeostasis and could therefore be involved in vascular plaques instability and calcification, thus, VDR polymorphisms may be associated with different risk for CVDs.[6-9] The association of this polymorphism with CAD risk in

diabetic individual had not been extensively studied in India.

We aimed at studying the occurrence of FokI VDR gene polymorphism in exon 2 in cases of diabetic CAD and compare with non- diabetic CAD in Indian population. Our investigation showed the nonsignificant association between VDR (FokI) genotypes and CAD risk in diabetic patients the north Indian population. Previous investigations of VDR polymorphisms and diabetes risk by other groups have produced inconsistent results.[11-14] In our study, allele F of Vitamin D receptor gene constituted 66 %(n=53) of total gene pool in CAD with DM patients, 61 %(n=49) in CAD without DM patients. The difference was found to be not significant between the CAD with DM and CAD without DM. (OR-1.24, 95% CI: 0.65- 2.3)

Allele f of Vitamin D receptor gene constituted 34 %(n=27) of total gene pool in CAD with DM patients, 39 %(n=31) in CAD without DM patients. The difference was found to be not significant between the two groups. (OR-0.80, 95% CI: 0.42-1.5)

CONCLUSION

Serum Vitamin D levels were decreased in both groups of patients, more significantly decreased in the presence of DM in CAD patients. Serum IL-4 levels were significantly higher in CAD with DM group as compared to CAD without DM group. No associations could be found between Vitamin D receptor gene FokI polymorphism and risk of CAD in diabetic and non-diabetic individuals. No significant correlation was found between vitamin D and IL-4 levels in the patients of both groups. No significant association was observed between low 25-hydroxy vitamin D levels with VDR FokI genotypes in both groups of patients. The association between VDR FokI polymorphism, vitamin D and inflammatory markers needs to be further explored in diabetic CAD patients. A bigger study involving a much larger number of patients must be conducted to generalize the results of this study.

REFERENCES

1. Prasad DS, Kabir Z, Dash AK, Das BC. Cadiovascular risk factors in developing countries: A review of clinico-epidemiological evidence. CVD Prev Control. 2010;5:115–23.
2. GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015; 385:117.
3. Rapsomaniki E, Timmis A, George J, et al. Blood pressure and incidence of twelve cardiovascular diseases: lifetime risks, healthy life-years lost, and age-specific associations in 1·25 million people. Lancet 2014; 383:1899.
4. Libby P. Inflammation in atherosclerosis. Arterioscler Thromb Vasc Biol. 2012;32(9):2045–51.
5. Rodondi N, Marques-Vidal P, Butler J, et al. Markers of atherosclerosis and inflammation for prediction of coronary heart disease in older adults. Am J Epidemiol. 2010;171:540–49.
6. Muscogiuri G, Nuzzo V, Gatti A, et al. Hypovitaminosis D. A novel risk factor for coronary heart disease in type 2 diabetes? Endocrine. 2016;51:268–73
7. Reid IR, Bolland MJ. Role of vitamin D deficiency in cardiovascular disease. Heart. 2012;98:609–14.
8. Jablonski KL, Chonchol M, Pierce GL, et al. 25-Hydroxyvitamin D deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults. Hypertension. 2011;57:63
9. Arababadi MK, Hassanshahi G, Azin H. Association of intron 8 but not exon 9 VDR polymorphisms with multiple sclerosis in south-eastern Iranian patients. Arch Iran Med. 2010;43:67-98.
10. Garcia D, Angel B, Carrasco E. VDR polymorphisms influence the immune response in type 1 diabetic children from Santiago, Chile. Diabetes Res Clin Pract. 2007;77:134–140.
11. Boraska V, Skrabic V, Zeggini E. Family-based analysis of vitamin D receptor gene polymorphisms and type 1 diabetes in the population of South Croatia. J Hum Genet. 2008;53:210–214.
12. Al Mheid I, Patel R, Murrow J, et al. Vitamin D status is associated with arterial stiffness and vascular dysfunction in healthy humans. J Am Coll Cardiol. 2011;58:186–92.
13. Dilmec F, Uzer E, Akkafa F, Kose E, van Kuilenburg AB. Detection of VDR gene ApaI and TaqI polymorphisms in patients with type 2 diabetes mellitus using PCR-RFLP method in a Turkish population. J Diabetes Complications. 2010 May-Jun;24(3):186-91. doi:10.1016/j.jdiacomp.2008.12.002. Epub 2009 Jan 30.
14. Malecki MT, Frey J, Moczulski D, Klupa T, Kozek E, Sieradzki J. Vitamin D receptor gene polymorphisms and association with type 2 diabetes mellitus in a Polish population. Exp Clin Endocrinol Diabetes. 2003 Dec;111(8):505-9.
15. Bid HK, Konwar R, Aggarwal CG, Gautam S, Saxena M, Nayak VL, Banerjee M. Vitamin D receptor (FokI, BsmI and TaqI) gene polymorphisms and type 2 diabetes mellitus: a North Indian study. Indian J Med Sci. 2009 May;63(5):187-94. doi: 10.4103/0019-5359.53164.
16. Kendrick J., Targher G., Smits G., Chonchol M. 25-Hydroxyvitamin D deficiency is independently associated with cardiovascular disease in the third national health and nutrition examination survey. Atherosclerosis. 2009;205:255–260. doi: 10.1016/j.atherosclerosis.2008.10.033.
17. Petimar J, Smith-Warner SA, Fung TT, Rosner B, Chan AT, Hu FB, et al. Recommendation-based dietary indexes and risk of colorectal cancer in the Nurses' Health Study and Health Professionals Follow-up Study. Am J Clin Nutr. 2018 Nov 1;108(5):1092-1103. doi: 10.1093/ajcn/nqy171.
18. Kim D.H., Sabour S., Sagar U.N., Adams S., Whellan D.J. Prevalence of hypovitaminosis D in cardiovascular diseases (from the national health and nutrition examination survey 2001 to 2004) Am. J. Cardiol. 2008;102:1540–1544. doi: 10.1016/j.amjcard.2008.06.067
19. Zittermann A. Vitamin D and cardiovascular disease. Anticancer Res. 2014;34:4641–48.
20. Mcgreevy C, Williams D. New insights about vitamin D and cardiovascular disease: A narrative review. Ann Intern Med. 2011;155:820–26.
21. Scragg R., Jackson R., Holdaway I.M., Lim T., Beaglehole R. Myocardial infarction is inversely associated with plasma 25-hydroxyvitamin D3 levels: A community-based study. Int. J. Epidemiol. 1990;19:559–563. doi: 10.1093/ije/19.3.559.
22. Cigolini M, Iagulli MP, Miconi V, Galiotto M, Lombardi S, Targher G. Serum 25-hydroxyvitamin D3 concentrations and

prevalence of cardiovascular disease among type 2 diabetic patients. Diabetes Care 2006;29:722–724
23. Huang H, Lavoie-Lamoureux A, Moran K, Lavoie JP. IL-4 stimulates the expression of CXCL-8, E-selectin, VEGF, and inducible nitric oxide synthase mRNA by equine pulmonary artery endothelial cells. Am J Physiol Lung Cell Mol Physiol. 2007 May; 292(5):L1147-54.
24. Zhao G, Dharmadhikari G, Maedler K, Meyer-Hermann M. Possible role of interleukin-1β in type 2 diabetes onset and implications for anti-inflammatory therapy strategies. PloS Comput Biol. 2014;10:e1003798. doi: 10.1371/journal.pcbi.1003798.
25. Lee YW, Kim PH, Lee WH, Hirani AA. Interleukin-4, Oxidative Stress, Vascular Inflammation and Atherosclerosis. Biomol Ther (Seoul). 2010 Apr;18(2):135-144.
26. King VL, Szilvassy SJ, Daugherty A. Interleukin-4 deficiency decreases atherosclerotic lesion formation in a site-specific manner in female LDL receptor−/− mice. Arterioscler. Thromb. Vasc. Biol. 2002;22:456–461.

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