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Association of CYP24A1 gene, Vitamin D deficiency and heart diseases in Pakistani patients

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Abstract

To analyze the association of CYP24A1 gene polymorphism with vitamin D deficiency and its related cardiovascular disorders in the Pakistani population. The present prospective cohort study was carried out at KRL and Rawalpindi General Hospital from January 2017 to December 2018. A total of 110 subjects suffering from heart diseases like hypertension, myocardial infarction, or congestive cardiac failure (age range 12-90 years) were enrolled in this study with informed consent. Sampling was done by non-probability convenience sampling. Blood 24Hydroxyvitmin D levels were assessed in the participants by using an electrochemiluminescence system. Genetic polymorphism in the CYP24A1 gene was screened in selected patients (n=15) through PCR-RFLP, after genomic DNA extraction from the whole blood. Data were analyzed using SPSS version 24 for Mac. Fisher's exact test and chi-square tests were applied for qualitative variables. The risk of polymorphism in CYP24A1(SNP rs6013897) genotypes TT, AT, and AA was determined by calculating the odds ratio and confidence interval. P-value <0.05 was considered statistically significant. We observed the highest level of vitamin D deficiency in patients of age group 18-45 years and insufficiency in age group 50-60years. A greater percentage of female patients (34.19%) were deficient in Vitamin D as compared to males (25.72%). Vitamin D deficiency was found to be associated with cardiovascular diseases like hypertension (P=0.000*), myocardial infarction (P=0.334), and heart failure (0.001*). CYP24A1 (SNP rs6013897) was significantly associated (P=0.000*) with vitamin D deficiency and heart diseases (P=0.004*). Moreover, significant polymorphism of genotype AT was observed in our subjects. {P=0.007*, Cramer's V =.003 and 95% CI (0.44-17.27)}. There is an association of CYP24A1 gene polymorphism with vitamin D deficiency and heart diseases. Prevention of low vitamin D levels in individuals with this known genetic marker may help to avert the development of heart diseases in Pakistan.

Keywords: vit D deficiency, cardiovascular diseases, CYP24A1 gene, polymorphism

1. Introduction

The steroid hormone Vitamin D is found to have a variety of functions in humans. The receptor (VDR) of an active form of Vitamin D is also present in vascular smooth muscle cells and cardiomyocytes (1). In recent research, the deficiency of Vitamin D is found to be associated with cardiovascular diseases. Recently vitamin D metabolism has been found to modulate many cardiovascular functions. The low serum levels of vitamin D are associated with hypertension, coronary artery disease (CAD), and heart failure (HF) (2).

This is very strange that despite high levels of sunshine, in Pakistan high levels of deficiency are found among all age groups, genders, income levels, and locations. There is a need for public health strategies to decrease the deficiency rate by food fortification and increasing exposure to sunlight (3).

According to American guidelines serum 25-hydroxyvitamin D levels are labeled a) deficient, when below or equal to 20 ng/mL, b) insufficient, when between 20 and 30 ng/mL, and c) normal when greater than 30 ng/mL (4).

The cause of vitamin D deficiency may be related to sun exposure, diet, and its absorption from the intestines. In Pakistan, this may be due to an indoor lifestyle (sun deprivation) especially in females, use of sunscreens, advanced age, air pollution, smoking, poor food absorption (malabsorption syndromes), Kidney or liver diseases, and drugs like anticonvulsants or glucocorticoids (3). Vitamin D deficiency is associated with hypertension. Its mechanism of action is by renin gene expression which increases the synthesis of renin leading to hypertension (5).

CAD has been associated with vitamin D deficiency its cause may be due to the presence of VDR in both the myocardium and vascular cells. In cases of Myocardial infarction, Vitamin D deficiency is found to be very common and the prognosis of the disease depends on the level of its deficiency (5).

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HF has been associated with vitamin D deficiency. A high prevalence of vitamin D deficiency is found in patients with HF, and it was inversely correlated with left ventricular function and severity of disease (5).

The gene CYP24A1encodes is a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenase which is responsible for catalyzing several reactions in the synthesis of cholesterol, steroids, lipids, drug metabolism, and it is found to regulate the level of vitamin D3. The genetic polymorphism of CYP24A1, hydroxylase-enzyme is suspected to be responsible for the inactivation of vitamin D (6).

Alternatively, spliced transcript variants encoding different isoforms have been found for this gene. 1,25-dihydroxyvitamin D3 is the physiologically most active form of vitaminD3 which binds to the vitamin D receptor. Inactivation of Vitamin D: Several hydroxylation steps occur in the catabolism of 1,25-dihydroxyvitamin D3 to calcitroic acid. The first of these steps are catalyzed by the enzyme 1,25-hydroxyvitamin-D3-24-hydroxylase, which is encoded by the CYP24A1 gene (7).

Our objective was to identify genetic variants responsible for the variation in serum 25(OH)D levels The inactivation of vitamin D metabolites relies upon two pathways which both include steps catalyzed by 1,25-hydroxyvitamin-D3-24-hydroxylase; CYP24A1 encodes this mitochondrial enzyme which is part of the cytochrome P450 system. We hypothesized that candidate gene CYP24A1, functionally important for vitamin D metabolism and pathways, must be responsible for variation in serum 25(OH)D levels.

2. Materials and Methods

In the present prospective cohort study, a total of 110 male and female subjects from the age range (12-90 years) were included. This was conducted from January 2017 to December 2018. 110 patients, male (40) and female (70) age range 20–60 years were recruited from KRL hospital and Rawalpindi General hospital. we randomly sampled patients. Of the 150 participants selected for this study, 30 (20%) were excluded because the serum 25(OH)D level could not be measured or was below the lower detection limit, and 10 (6.6%) were excluded because less than 95% of the markers were successfully genotyped across all the SNPs. This exclusion process left 110 participants (70 women and 40 men) for analysis. The clinical characteristics and mean laboratory values of the 70 women and 40 men are shown in Table 1, Fig 1. Vitamin D (deficient) are patients with vitamin D levels<25 ng/ml. Vitamin D (insufficient) with vitamin D levels from 25-32ng/ml and vitamin D normal with levels between 32-80 ng/ml

All the subjects included in the present study had been admitted to the hospital for cardiovascular diseases like hypertension, Myocardial infarction, or congestive heart failure. The subjects were excluded from the study if they had diseases deemed to affect vitamin D metabolisms, such as cancer, hyperthyroidism, diabetes mellitus, primary hyperparathyroidism, pituitary, or adrenal and rheumatic diseases. Participants who had taken vitamin D and/or calcium supplements within the past 3 months were also excluded. After these exclusions, 110 participants entered the study. The study was approved by the Ethics Committee of the KRL hospital. All the participants signed informed consent forms before entering the study. Vitamin D (deficient) are patients with vitamin D levels<25 ng/ml. Vitamin D (insufficient) with vitamin D levels from 25-32ng/ml and vitamin D normal with levels between 32-80 ng/ml.

2.1. Measuring serum 25(OH)D

The serum levels of 25(OH)D were determined using an automated Roche electrochemiluminescence system (E170; Roche Diagnostic GmbH, Mannheim, Germany). The intraassay coefficients of variation (CVs) for 25(OH)D were 5.7% at a level of 25.2 ng/mL, 5.7% at a level of 39.9 ng/ml, and 5.4% at a level of 65.6 ng/mL, respectively. The interassay CVs for 25(OH)D were 9.9% at a level of 25.2 ng/mL, 7.3% at a level of 39.9 ng/ml, and 6.9% at a level of 65.6 ng/mL, respectively. The lower detection limit of 25(OH)D was 4ng/mL. Blood 24Hydroxyvitmin D levels were assessed in the participants.

Genetic polymorphism in the CYP24A1 gene was screened in selected patients (5 male) and (10 female) through PCR-RFLP, after genomic DNA extraction from the whole blood. DNA was extracted from peripheral blood samples through QIAamp DNA mini kit (Qiagen) according to the manufacturer's protocol and was quantified using an ND-1000 spectrophotometer (NanoDrop; Thermo Fisher Scientific Inc). The polymorphism in CYP24A1(rs6013897) was amplified using the primer's 5'-CTTGATCCAATGTCCGCAC-3' (forward) and 5'-CTTTGGGTAGGTTACTTCGC-3' (reverse). The amplicons were electrophoresed on 2% agarose gel stained with ethidium bromide and were visualized under UV light. The PCR products were purified using FastAP Thermosensitive Alkaline Phosphatase (Thermo Fisher Scientific Inc.) and exonuclease 1(Fermantas). The samples were placed in a 96-well optical plate and sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit and Big Dye Xterminator Purification kit. The plate was then placed in a 3500xL Genetic Analyzer (Applied Biosystems Inc.) for electrophoresis. The products of sequencing were visualized, and results were interpreted using the Sequence Scanner Software v1 (Applied Biosystems). `Cytogenetic Location: 20q13.2, which is the long (q) arm of chromosome 20 at position 13.2 and Molecular Location: base pairs 54,145,731 to 54,174,032 on chromosome 20.

Data were analyzed using SPSS version 24 for Mac. Pearson's coefficient for correlation was calculated for 25(OH)D levels with age and gender. Fisher's exact test and

chi-square test were applied for qualitative variables. The risk of polymorphism in CYP24A1(SNP rs6013897) genotypes TT, AT and AA was determined by calculating the odds ratio at the 95%confidence interval. A P value <0.05 was considered statistically significant.

3. Results

The study included 110 participants (70 women and 40 men) for analysis. The clinical characteristics and mean laboratory values of the 70 women and 40 men are shown in Table 1, Fig 1. Vitamin D (deficient) are patients with vitamin D levels<25 ng/ml. Vitamin D (insufficient) with vitamin D levels from 25-32ng/ml and vitamin D normal with levels between 32-80 ng/ml.

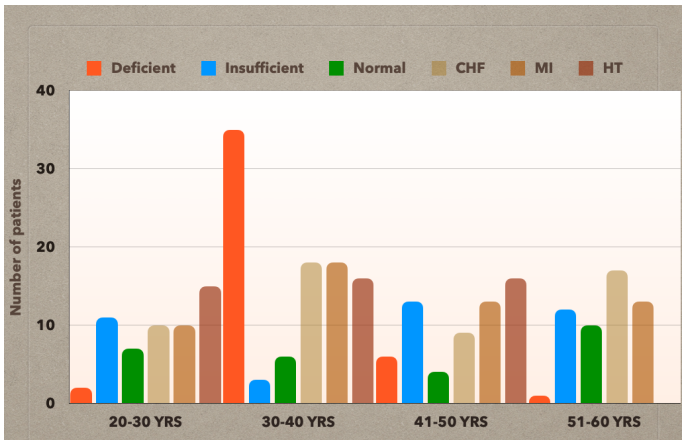

Fig. 1. Distribution of patients of different age groups with congestive heart failure (CHF), myocardial infarction (MI), hypertension (HT), Vit D deficiency, insufficiency, and normal levels.

Generally, the men had higher serum 25(OH)D levels than the women, and old age was significantly associated with its deficiency. By Pearson correlation analysis, 25(OH)D levels showed a significant correlation with age (r = -0.206*; p < 0.05) and gender (r=-0.579*; P<0.001). We observed the highest level of vitamin D deficiency in patients of age group 18-45 years and insufficiency in age group 50-60 years as shown in Fig 1.

A greater percentage of female patients (34.19%) were deficient in Vitamin D as compared to males (25.72%) shown in Fig 2. Vitamin D deficiency was found to be associated with cardiovascular diseases like hypertension (P=0.000*), myocardial infarction (P=0.334), and heart failure (0.001*). CYP24A1 (SNP rs6013897) was significantly associated (P=0.000*) with vitamin D deficiency and heart diseases (P=0.004*).

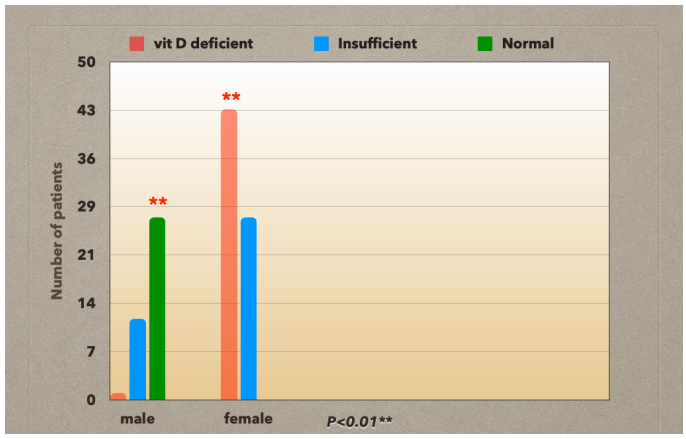

Fig. 2. Distribution of Vit D deficient, insufficient and normal males and females with heart diseases

Moreover, significant polymorphism of genotype AT was observed in our subjects. {P=0.007*, Cramer's V =.003 and 95% CI (0.44-17.27)} Table 1, 2 and 3.

Table 1. The polymorphism of CYP24A1(rs 6013897) genotypes TT, AT, and AA is associated with the risk of Vit D deficiency and heart diseases

CYP24A1	TT			AT			AA		
SNP(rs 6013897)	P	CV	95% CI	P	CV	95% CI	P	CV	95% CI
Vit D deficiency	0.667	0.189	0.904-1.366	0.007**	0.756	1.44-17.27	0.667	0.189	0.904-1.366
Heart diseases	0.73	0.53	0.913-1.32	0.026*	0.013	1.39-9.62	0.73	0.53	0.913-1.326

Fisher,s exact test P<0.001**, CV=Cramer's V, Odds ratio=OR, 95% CI= Confidence interval

Table 2. The risk of association of CYP24A1 gene and Vitamin D deficiency in patients of heart diseases like MI, HT, and CHF

Heart Diseases												
	MI				HT				CHF			
CYP24A1	P	CV	OR	95% CI	P	CV	OR	95% CI	P	CV	OR	95% CI
	0.58	0.264	4.0	0.323-49.5	**0.047***	0.57	16.0	1.09-234.2	0.667	0.464	1	0.904-1.366
Vit-D deficiency	0.294	0.264	4.0	0.323-49.5	**0.047***	0.025	16	1.093-234.2	0.667	0.464	1	0.904-1.366

Table 3. Correlation of Heart disease, CYP24A1 gene, and Vitamin D deficiency in patients

Correlations				
		Heart disease	CYP24A1	Vit D (deficient)<25 ng/ml
Heart disease	Pearson Correlation	1	.853**	.853**
	Sig. (2. tailed)		0.000	0.000
CYP24A1	Pearson Correlation	.853**	1	1.000**
	Sig. (2. tailed)	0.000		0.000
Vit D (deficient)<25 ng/ml	Pearson Correlation	.853**	1.000**	1
	Sig. (2. tailed)	0.000	0.000	

**Correlation is significant at the 0.01 level (2-tailed)

4. Discussion

There is an association between Vitamin D deficiency, cardiovascular diseases, and the CYP24A1 gene. The Vitamin D endocrine system is essential for calcium homeostasis, and low levels of vitamin D metabolites are associated with cardiovascular disease risk. CYP24A1 is an important gene which encodes 1, 25-dihydroxyvitamin D3 24-hydroxylase enzyme. This enzyme degrades the hormonally active form of vitamin D, Calcitriol, and its precursor Calcidiol. Calcitriol has anti-proliferative and pro-apoptotic functions. Genetic polymorphism at key positions in CYP24A1 has shown an association with deficient levels of vitamin D and related diseases. The study confirms the association of CYP24A1 gene polymorphism with vitamin D deficiency and its related cardiovascular disorders in the Pakistani population.

Different studies have implicated a deficient level of Vitamin D in cardiovascular diseases, including coronary artery disease. Currently, the metabolism and homeostasis of Vit D have garnered a lot of interest, and research is being carried out to observe the benefit of this vitamin in the cardiovascular system. Since the hydroxylase enzyme which causes the inactivation of Vit D is controlled by gene expression, so the effect of genetic polymorphism rs2762939 of CYP24A1 in modifying the inactivation of vitamin D, is under consideration (8). It has been reported that the loss of CYP24A1 function resulted in an increased serum concentration of 1,25-dihydroxyvitamin D (9).

For the control of Vit D level, the CYP24A1A gene is the third cytochrome P-450 gene. This gene, located on chromosome 20, at 20q13.2-q13.3, spanning 20.53 kb on the reverse strand, carries the code for the 1a,25(OH)2D inactivation protein (10).In a study conducted on a family using Terminal deoxynucleotidyl Transferase (TdT), an intronic SNP, rs17219315, was found to be associated with 25(OH)D levels (11). However, in another study, no significant association was observed between CYP24A1 and serum 25(OH)D concentration (12). In addition, CYP24A1 polymorphisms were associated with many diseases, such as stroke and hypertension. Wei Yang et al. reported that CYP24A1 rs1570669 was linked to a reduced risk of stroke, and rs6068816 could increase susceptibility to ischemic stroke indicating that in the Chinese population CYP24A1 gene polymorphism is associated with heart diseases (13).

In our study, we found an association of vitamin D deficiency with hypertension which is due to the involvement of the renin-angiotensin-aldosterone system (RAAS). The juxtaglomerular cells of the kidney produce and release renin, which then brings about the conversion of Angiotensin I to Angiotensin II, ultimately resulting in increased secretion of aldosterone. Angiotensin has a direct effect on increasing blood pressure by its vaso-constrictor activity. Its indirect effect in causing an increase in blood pressure is due to its salt and water retaining activity. Research using VDR and 1α-hydroxylase knockout mice have described abnormally increased activity of the renin-angiotensin-aldosterone system. Vitamin D has been reported to cause inhibition of the gene for renin production, thus resulting in reduced activity of this system (14).

Furthermore, an association of vitamin D deficiency with hypertension was also observed and an inverse association was observed between serum levels of 25-hydroxyvitamin D and Blood Pressure (15,16). Some prospective studies have also been conducted to assess the association of vitamin D with variations in BP as well as the development of hypertension. In Groningen, Holland, Van Ballegooijen et al. (2015), measured vitamin D levels of about five thousand normotensive people, who were then followed up for almost six and a half years. At the end of the study period, 20% of the participants with low levels of vitamin D developed hypertension, thereby indicating an increased risk of development of increased BP in individuals with low levels of this vitamin. (17)

In our study, we found an association of myocardial infarction with reduced vitamin D levels. This is understandable, considering the presence of vitamin D receptors in the heart muscle as well as vascular cells. Different epidemiological studies have reported increased occurrence of coronary artery disease and vitamin D deficiency in countries located away from the equatorial regions and in wintertime when exposure to sunlight is limited (18). Studies have also proposed a likely association of vitamin deficiency with the immediate and long-term prognosis of acute myocardial infarction (MI) as this deficiency was observed quite frequently in patients of acute myocardial infarction (AMI) (19). Additionally, an association of vitamin D levels with the number of coronary vessels affected is the reason claimed for observance of complications associated with MI as well as a repetition of unfavorable cardiac outcomes, etc., in people with low Vit D (18).

The Health Professionals Follow-up Study observed about eighteen thousand males for ten years and noted a positive correlation of reduced vitamin D levels with raised AMI risk, keeping in mind various risk factors (19) Furthermore, prospective studies have reported a high occurrence of vitamin D deficiency in individuals admitted to the hospital for acute MI. A multicenter study on two hundred and thirty-nine patients suffering from acute coronary syndrome indicated that 96% of them suffered reduced vitamin D levels at the time of admission to the medical care facility. (20). In patients hospitalized with Acute Coronary Syndrome (ACS), the intra-hospital death rate is seen to be associated with serious vitamin D deficiency. In a study (21) the patients of ACS observed a 24% rate of intra-hospital cardiovascular mortality when vitamin D levels fell to below 10 ng/mL. This mortality rate was appreciably greater than that observed in the remaining patients in whom it was only 4.9% (21).

Even though research has shown a link between vitamin D

and Heart Failure (HF), the precise mechanism using which, a deficient level of this vitamin results in poor clinical prognosis, is unsure. A likely mechanism suggested is the development of cardiorenal syndrome or deterioration in kidney function (22). The cardiovascular and renal systems being inextricably linked affect the activity of the other. Increased sympathetic stimulation, systemic inflammation, and up-regulated activity of the Renin-Angiotensin-Aldosterone System (RAAS) lead to worsening of the cardiorenal syndrome resulting in an imbalance of water and electrolytes, endothelial dysfunction, and possibly leading to left ventricular remodeling and myocardial fibrosis. The result is a positive feedback mechanism that leads to a further worsening in the functioning of these systems (23). The deficiency of vitamin D may result in amplification of the inflammatory response leading to increased cytokine production. This may exert a damaging effect on cardiac muscle by promoting cell hypertrophy, apoptosis, and replacement by fibrous tissue, a reduction in force of contraction of the heart, as well as causing fibrosis and failure of the kidneys (24).

There is an association between vitamin D deficiency, cardiovascular diseases, and the CYP24A1 gene. This finding may help to prevent and treat such cardiovascular disorders by identifying the high-risk individuals, with known genetic markers of vitamin D deficiency of the Pakistani population. As the incidence of cardiovascular diseases is increasing in the Pakistani population, a greater focus will be needed to better elucidate the role of vitamin D in the pathogenesis of symptoms. Cardiovascular diseases remain the main cause of mortality in several countries worldwide. An understanding of the pathophysiological mechanisms involved, as well as their risk factors, is essential for the planning of prevention and treatment strategies. A better understanding of the genetic involvement of VDR gene polymorphisms in the regulation of vitamin D metabolite concentrations from further studies may have important implications in the use of the genetic profile to identify individuals who may be at risk for deficiency of vitamin D.

Conflict of interest

None to declare.

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