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**Independent assortment of *GC* gene polymorphism (rs2282679) and 25-hydroxyvitamin D levels in
Coronary Artery Disease**

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Abstract

BACKGROUND: Coronary artery disease (CAD) remains a major public health burden. Emerging research has suggested an association between vitamin D insufficiency and CAD. Vitamin D binding protein (VDBP) is the primary vitamin D carrier and many of its genetic polymorphisms are able to induce the expression of proteins with different affinities for the vitamin, which in turn, might affect its serum levels and CAD incidence.

SUBJECTS/METHODS: 112 male patients, aged between 35 and 50, with verified CAD and 109 age- and sex-matched controls were recruited. Genotyping was performed by the TaqMan allelic discrimination assay and plasma 25(OH)D levels were assessed by HPLC-UV. Serum parathyroid hormone (PTH) and VDBP levels were measured using ELISA.

RESULTS: s-25(OH)D levels in CAD patients were significantly lower than the controls. Whereas, s-PTH levels were significantly higher in the CAD patients than in controls. There was no significant difference in the distribution of *GC* genotypes among both groups. s-25(OH)D showed a weak inverse correlation with s-PTH levels.

CONCLUSIONS: Serum levels of vitamin D and PTH are highly correlated to CAD incidence. However, s-VDBP level is neither associated with disease outcome nor with vitamin D status. *GC* gene variant has no effect on 25(OH)D levels.

Keywords: Vitamin D – 25(OH)D - Vitamin D binding protein (VDBP) – SNPs - Parathyroid hormone (PTH) – Coronary artery disease (CAD)

Introduction

Cardiovascular disease (CVD) is considered one of the top most serious health problems in Egypt and worldwide. Numerous risk factors are linked with the incidence of CVD; many of them are traditional risk factors such as diabetes mellitus, hypertension and obesity. Others are non-traditional due to immune-

deficiency problems, malnutrition and vitamin deficiencies including low vitamin D levels (Holick and Chen (2008)). The deep-rooted relation between vitamin D deficiency (VDD) and CVD has been readily established in recent studies. This can be attributed but not limited to the beneficial role of vitamin D in modulating vascular inflammation (Rigby et al. 1987), platelet aggregation/thrombogenesis (Aihara et al. 2004), vascular smooth muscle cell proliferation (Mitsubishi et al. 1991), the renin-angiotensin system (Li et al. 2003), cardiomyocyte proliferation (Artaza et al. 2009), vascular calcification, myocardial fibrosis and proliferation (Artaza et al. 2009).

Over the years, 25(OH)D has been chosen as a gold standard in vitamin D assays that can provide accurate indication of the vitamin stores, as it possesses a long half-life and its production by the liver depends mainly on the vitamin concentration and isn't significantly regulated by other parameters (Clemens et al. 1986). It has been also estimated that up to 90% of this 25(OH)D is carried on vitamin D binding/transporting protein (VDBP) in the blood stream, thus spawning a perception of the use of VDBP as a key determinant of 25(OH)D levels (Ku et al. 2013). Furthermore, VDBP is found to be specifically responsible for vitamin D endocytosis (Nykjaer et al. 1999).

VDBP is encoded by the *GC* gene which is located on Chromosome 4q13. Two non-synonymous SNPs in exon 11, namely, rs7041 (*Gc1*) and rs4588 (*Gc2*) gave rise to three distinct gene products that differ in their binding affinity to vitamin D (Speeckaert et al. 2014). To date, most studies have proposed SNPs of the *GC* gene as predictors of 25(OH)D concentrations, but not disease incidence. For instance, Kühn and his coworkers reported an association between two SNPs in the *GC* gene (rs1155563 and rs2282679) and 25(OH)D levels, but not with CVD incidence (Kühn et al. 2013).

The non-coding SNP, rs2282679 in intron 12, was found to be in tight linkage disequilibrium with rs4588 ($r^2=1.0$ in HapMap-CEU panel and 0.7 in HapMap-MEX panel). It has also been less well studied as a potential contributor to variation of VDBP or 25(OH)D levels and incidence of CAD, compared to both rs7041 and rs4588 SNPs. None have been tested in the Egyptian population.

Previous studies demonstrated association between SNP rs2282679 and some diseases. The recessive model of SNP rs2282679 in *GC* gene was found to be significantly associated with the occurrence of hip fracture in Japanese patients with rheumatoid arthritis through a 10-year follow-up (Yoshida et al. 2014). The

SNP rs2282679 also proved to be associated with Parkinson's disease progression and serum 25OHD levels in a Turkish cohort study (Gezen-Ak et al. 2017). Literature search also pointed out to the existence of a strong relationship between VDBP and Alzheimer's disease (AD) pathology. VDBP was found with high levels in the CSF of patients with AD (J. Zhang et al. 2008). Lauren Mokry et al. proposed the SNP rs2282679 as a strong independent predictor of AD (Mokry et al. 2016).

One of the prime targets of $1,25(\text{OH})_2\text{D}_3$ (the active form of vitamin D) is the parathyroid gland where it plays a crucial role in regulating the production and secretion of PTH (Demay et al. 1992; Silver & Naveh-Manly (2010)). Vitamin D therapies also proved to be highly successful in the suppression of secondary hyperparathyroidism (Silver & Naveh-Manly (2010); Slatopolsky et al. 2005). And, in a study done in U.S, PTH levels showed an inverse relation with $25(\text{OH})\text{D}_3$, with a delay of 4 weeks among all genders and latitudes (Kroll et al. 2015).

Broadly speaking, there is a growing body of evidence supporting the role of vitamin D apace with parathyroid hormone (PTH) in chronic heart diseases (Gruson et al. 2015). Experimental and clinical studies provided many clues that increased PTH levels above its normal range may be the reason behind or at least contributes to vascular diseases such as endothelial dysfunction, increased vascular stiffness, hypertension and pre-cerebral artery atherosclerosis (Perkovic et al. 2003). Additionally, in their study on healthy Icelandic adults, Steingrimsdottir and his collaborators predicted the existence of an inverse relationship between $s\text{-}25(\text{OH})\text{D}$ and $s\text{-PTH}$ levels and which was found to be independent of calcium intake (Steingrimsdottir et al. 2005). Furthermore, in a study done by Emil Hagström et al on two independent community-based Cohorts; increased PTH was highly linked to the degree of atherosclerosis and the risk of atherosclerotic disease (Hagström et al. 2014). Some researchers mentioned that higher PTH levels can contribute to atherogenesis by two mechanisms; It either exerts its action directly by binding to PTH receptors on the vessel wall causing vascular calcification and vascular remodeling or indirectly by inducing inflammation and vascular dysfunction (Walker et al. 2009). Owing to all the aforementioned, the present study evaluated the influence of the *GC* polymorphism rs2282679 on $s\text{-}25(\text{OH})\text{D}$, $s\text{-VDBP}$, $s\text{-PTH}$ levels and on the early incidence of CAD in the Egyptian population.

Subjects and Methods

Study population

112 male patients aged between 35 and 50 years were recruited (in- and out-patients) from the National Heart Institute (NHI) in Imbaba and from El-Kasr El-Einy hospital, Cairo, Egypt. They had either a history of myocardial infarction, percutaneous coronary intervention, or coronary catheterization-verified coronary artery disease (CAD). In our country, recent research has mentioned that Multivessel disease was more frequent among males ($p=0.043$) and smokers ($p=0.020$) (Fawzy et al. 2017). Also, Blanton et al mentioned that VDBP levels were lower in males, than in females and relayed this to estrogen effects on VDBP (Blanton et al. 2011). So, in this study, we aimed at excluding the effect of sex on VDBP and vitamin D levels by including males only. Exclusion criteria also included any other acute or chronic disease such as kidney or liver diseases or diabetes mellitus. The blood pressure of the study patients was controlled ($<140/90$ mm/Hg). 109 sex and age matched healthy volunteers were also enrolled herein. The baseline characteristics for all study subjects are clearly mentioned in table 1.

Laboratory analyses

Blood samples were collected from all subjects involved in this study in EDTA vacutainers and centrifuged at 2,500 rpm for 15 minutes at 4°C . The resulting plasma was stored in a -80°C freezer until 25(OH)D analysis. Levels of total 25-hydroxyvitamin D (D_2 and D_3) were determined for both patients and controls using an in-house developed and validated high performance liquid chromatography with Ultraviolet detection method (HPLC-UV). Subjects identified as having normal/sufficient vitamin D levels had 25(OH)D concentrations greater than or equal to 30 ng/mL, whereas insufficient and deficient subjects had 25(OH)D concentrations 20-29 ng/mL and less than 20 ng/mL, respectively. *s*-VDBP levels were measured for both groups using a commercial enzyme-linked immunosorbent assay (R&D Systems) that uses two monoclonal antibodies in a sandwich format (interassay coefficient of variation, 7.2%). *s*-PTH levels were measured using Elecsys Parathyroid Hormone Immunoassay kit (Modular Analytics E170, Roche Diagnostics) for both patients and controls.

Single nucleotide polymorphism (SNP) selection and genotyping

Genomic DNA was extracted from whole blood samples of all subjects using Thermo Scientific Gene Jet whole blood genomic DNA purification mini kit (#ko781, lot number 00135002) following the manufacturer's instructions. Purified DNA was genotyped for rs2282679 in intron 12 of the *GC* gene. Genotyping was performed using the Taqman assay using the Analyzer Applied Bio system (CA, USA) for both patients and controls

Statistical analyses

The Hardy-Weinberg equilibrium (HWE) was tested using the goodness-of-fit chi-square test. Chi Square tests were performed to test if genotype frequencies in the population remained constant from generation to generation. Analyses were performed using GraphPad Prism 6 statistics software. Pearson correlation was used to test for the presence of correlation between *s*-PTH and *s*-25OHD or between *s*-25OHD and *s*-VDBP or between *s*-VDBP and *s*-PTH. Vitamin D metabolites, *s*-PTH and *s*-VDBP levels were calculated as mean $\pm$ SEM. Mann-Whitney T-test was used for two-group comparisons. Associations of the different genotypes of the rs2282679 with *s*-25(OH)D, *s*-PTH and *s*-VDBP concentrations were tested using Kruskal-Wallis test. One way Anova was used to detect the difference in PTH levels among the three vitamin D status groups and then post-hoc test was done to determine where differences exist. Similarly, one way Anova was also used to detect the difference in VDBP levels among the three vitamin D status groups. Statistical significance was defined at a p-value ≤ 0.05 .

Results

The total 25(OH)D, 25(OH)D₃ and 25(OH)D₂ levels were all found to be significantly lower in patients compared to controls ($p < 0.0001$), which in turn, clarifies the tenacious association between serum vitamin D metabolites levels and the occurrence of CAD. The intra- and inter-comparisons among groups also showed a significant difference between different vitamin D metabolites ($p < 0.0001$), figure 1.

The mean serum PTH levels significantly increased among CAD patients compared to controls who have no incidence of CAD or any chronic condition ($p < 0.0001$) as shown in figure 2. Thus, our study established a solid relation between the CAD tendency and the *s*-PTH levels. The study also aimed to assess

the relationship among both *s*-PTH levels and vitamin D status. Figure 3 clearly demonstrates this relation. A significant increase in *s*-PTH levels is observed among individuals having vitamin D deficiency or insufficiency. In the combined subjects, those who had sufficient levels of vitamin D ($>30\text{ng/ml}$), had also lesser *s*-PTH levels indicated by a mean of 128.2 pg/ml , P-value is estimated to be 0.005. While, those having deficient ($<20\text{ng/ml}$) or insufficient ($20\text{-}30\text{ng/ml}$) levels of vitamin D, had higher *s*-PTH levels determined by a mean of 188.9 pg/ml and 182.0 pg/ml , respectively. A weak inverse correlation also existed between *s*-PTH and *s*-25(OH)D levels indicated by spearman correlation coefficient $r = -0.3362$ as displayed by figure 4.

Some groundwork and experimentation are spurring that the *GC* polymorphisms are associated with race and ethnicity, resulting in differences in *s*-VDBP levels and binding affinity that affect the transport and metabolism of vitamin D and its metabolites (Xie et al. 2014). We hereby examined the effect of genetic variation in the *GC* gene rs2282679 on the *s*-VDBP, *s*-25(OH)D levels or CAD incidence in the Egyptian population. Unexpectedly, the SNP rs2282679 of the *GC* has shown to have no effect on any of the previously mentioned parameters. Only the wild type (TT genotype) demonstrated an additive effect on decreasing *s*-25(OH)D₂ concentrations, p-value = 0.02, figure 5.

The genotypes of the rs2282679 *GC* polymorphism were in Hardy–Weinberg equilibrium, p-value = 0.076. Both the genotypic and allelic distribution of rs2282679 among different vitamin D status subgroups of CAD patients and Controls are presented in table 2. Overall, the genotypic and allelic distributions are highly similar among both patients and controls groups. The TT genotype (wild type) is shown to be the most prevalent genotype among both groups, followed by the GT genotype (hetero-type). The mutant genotype (GG) doesn't appear in this population. Likewise, The T allele is shown to be the most predominant and widespread allele when compared to the G allele. Our observations showed that none of the genotypes or alleles of rs2282679 was found to significantly influence CAD incidence or the total 25OHD level, figure 6.

Additionally, the genotypes of rs2282679 SNP of *GC* gene are tested for their correlation with vitamin D metabolites among patients, controls and total subjects. The results are presented in table 3. P-values were calculated using Unpaired T-test. The test is important to show if there is any significant increase or decrease in the concentrations of any of the vitamin D metabolites with a certain genotype. None of these genotypes

had an effect on *s*-25(OH)D levels except for the wild type (TT genotype) which was remarkably associated with lower *s*-25(OH)D₂ concentrations in the total subjects, indicated by a p-value of 0.02, figure 5.

It's even more intriguing to know that the circulating VDBP level exhibited no observable association with the incidence of CAD or vitamin D status. The levels of VDBP among both patients and controls groups are clearly displayed by table 4. The mean *s*-VDBP levels were found to be 808 µg/ml and 859.8 µg/ml in the patients and the controls group, respectively. Thus, *s*-VDBP was shown to be a little bit lower in the patients, yet, no statistically significant difference is obtained, p-value = 0.4969.

By the same token, we compared *s*-VDBP levels among different groups with regards to their vitamin D status. Observably, *s*-VDBP level demonstrated a miniature increase in groups having sufficient vitamin D levels. However, this increase isn't considered statistically significant and we can conclude that *s*-VDBP levels did not affect vitamin D status among all recruited groups, figure 7. The rs2282679 polymorphism in the GC gene didn't exhibit a significant effect on *s*-VDBP levels. This can be easily interpreted from figure 8.

Discussion:

Multiple genetic and non-genetic factors can contribute to vitamin D deficiency. Recent genome wide association studies pointed out to the potential role of the genetic variants of proteins involved in the vitamin D pathway, whether synthesis, metabolism, transport or elimination, in the control of its circulating levels (Touvier et al. 2015). However, with very few exceptions, those studies have focused primarily on populations of European descent (Gozdzik et al. 2011). In this regards, further research is required to elucidate genetic predictors for vitamin D status, with the purpose of identifying groups at high risk for vitamin deficiency, as well as, identifying novel genetic biomarkers for diseases that result from its deficiency.

It is also noteworthy to mention that the mean serum 25(OH)D level, which is considered the most reliable blood indicator for vitamin D status, is relatively affected by genetic variations in GC gene (the gene encoding VDBP), which is widely studied in Western populations (Sinotte et al. 2009). The exact mechanism linking changes in GC affinity, its circulating levels and the circulating 25(OH)D levels is not yet established. Different physiologic and pathologic conditions can affect *s*-VDBP levels, which in turn may affect

circulating 25(OH)D levels. Overall, the only repeatedly observed associations were noticed between common *GC* SNPs and levels of 25(OH)D (Ahn et al. 2010; Zhou et al. 2012).

SNP rs2282679 of the *GC* gene didn't exhibit any remarkable effect on CAD incidence in the Egyptian population. The distribution was as follows; TT (wild type) 64.2% in the controls and 71.4% in patients, GT 35.8% in the controls and 28.6% in patients, and GG 0 % in both controls and patients. Similar genotype distribution of rs2282679 was observed among the African American population (84.5% for TT, 15.2% for GT and 0.2% for GG) (Signorello et al. 2011).

Moreover, our findings indicate the observed genotypes and alleles had no significant effect on *s*-25(OH)D₃ and total *s*-25(OH)D levels. Only the wild allele (TT) was accounted to be in charge of low *s*-25(OH)D₂ levels in the control group as well as in the total subjects. Similarly, Zhang et al. did not find a significant association of *GC*-rs2282679, rs4588, rs7041 with serum levels of 25(OH)D in a study of 506 Northeastern Han Chinese children recruited from outpatient clinic (Zhang et al. 2013). Contrarily, allele T of the SNP rs2282679 was reported to be significantly associated with low 25OHD levels (Mokry et al. 2015). Ahn et al. and Wang et al. in their genome wide association studies found that rs2282679 variants were associated only with lower serum 25(OH)D₃ levels in white Europeans (Ahn et al. 2010; Wang et al. 2010).

Dustin Blanton et al study on the national Americans demonstrated no association between *s*-VDBP and *s*-vitamin D levels in pooled subjects (healthy and diabetic individuals), as well as, demonstrating no significant effect of *GC* polymorphisms on serum VDBP levels (Blanton et al. 2011). Likewise, our data-analysis showed no significant difference in the *s*-VDBP levels among both CAD patients and control groups. Conjointly, no significant association between *s*-VDBP and *s*-25(OH)D concentration was observed and no considerable effect of rs2282679 polymorphism in the *GC* gene on the *s*-VDBP levels was detected as shown in figure 8.

Having known the role of PTH in inflammation and its actions on vessel walls causing vascular calcification and vascular remodeling (Walker et al. 2009), it was reasonable to postulate that elevated levels of PTH might be a major contributor to the risk of CAD. Our findings provided added evidence to the

previously stated postulations by detecting a significant booming in the mean serum PTH levels among patients having CAD. Moreover, our study shed light on the existence of a significant association of vitamin D deficiency with increased mean serum PTH levels in the total subjects. In the same manner, Pekkinen et al declared the existence of a significant negative correlation between serum 25(OH)D and PTH among Finnish children and adolescents aged 7-19 years after controlling for calcium intake (Pekkinen et al. 2014).

It has been frequently reported that vitamin D deficiency highly contributes to the incidence of CAD and that maintaining sufficient vitamin D levels is extremely important for cardiovascular function (Kendrick et al. 2009; Kim et al. 2008; Tarcin et al. 2009; Zittermann et al. 2008). The current study showed a compelling decrease in 25(OH)D levels among the patients group, therefore, providing a surplus support to the former research. Nevertheless, Despoina Manousaki et al. stated that there was no association between genetically lowered 25OHD levels and CAD in their study and relaying the previous associations to reverse causation (Manousaki et al. 2016).

Conclusion:

In a nutshell, Vitamin D deficiency and elevated PTH levels are associated with CAD incidence. A significant negative correlation exists between vitamin D status and PTH levels. SNP rs2282679 of the *GC* has no effect on serum total 25(OH)D levels and only the wild type (TT genotype) has an additive effect on decreasing *s*-25(OH)D₂ concentrations. Circulating VDBP level has no association with CAD incidence or vitamin D status. SNP rs2282679 of the *GC* isn't associated with mean serum VDBP levels and has no correlation with CVD incidence.

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Statement of competing interests:

The authors declare no potential conflict of interest.

Declaration:

The authors declare that the experiment has been reviewed and approved by German University in Cairo's ethics review committee, and that the subjects have given informed consent prior to participating in the study.

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Tables:

Table 1: Baseline characteristics and laboratory analysis of the study population

Study population	Gender	Age Group	Total 25OHD conc. (ng/ml) / Vitamin D status	PTH (pg/ml) $\pm$ SEM	VDBP (μ g/ml) $\pm$ SEM	BMI	Fasting blood sugar level (mg/dl)	Chronic conditions
Patients (n=112)	Male (n=112)	35 to 39 (n=24)	17.48 $\pm$ 2.1 vitamin D deficient	207.2 $\pm$ 47.14	870.4 $\pm$ 80.4	BMI > 30 (n=14) BMI: 25 - 29.9 (n=2) BMI: 18.5 - 24.9 (n=8)	< 100 mg/dl (n=20) 100 - 125 mg/dl (n=4)	MI (n=20) Obesity (n=14)
		40 to 44 (n= 19)	16.05 $\pm$ 3.25 vitamin D deficient	179.2 $\pm$ 39.67	756.4 $\pm$ 87.25	BMI > 30 (n=3) BMI: 25 - 29.9 (n=6) BMI: 18.5 - 24.9 (n=10)	< 100 mg/dl (n=17) 100-125 mg/dl (n=2)	MI (n=11) Obesity (n=3)
		45 to 50 (n= 69)	21.08 $\pm$ 2.02 vitamin D insufficient	195.3 $\pm$ 24.62	844.9 $\pm$ 103.4	BMI > 30 (n=3) BMI: 25 - 29.9 (n=33) BMI: 18.5 - 24.9 (n=33)	< 100 mg/dl (n=45) 100-125 mg/dl (n=24)	MI (n=67) Obesity (n=3)

Controls (n=109)	Male (n=109)	35 to 39 (n=63)	69.13±3.98 vitamin D sufficient	109.3±5.55	886.0± 62.06	BMI: 25 - 29.9 (n=8) BMI: 18.5 - 24.9 (n=47) BMI < 18.5 (n=8)	< 100 mg/dl (n=109)	No chronic condition
		40 to 44 (n=6)	80.2 ± 12.61 vitamin D sufficient	117.29± 4.3	998.9±70.53	BMI: 25 - 29.9 (n=2) BMI: 18.5 - 24.9 (n=4)		
		45 to 50 (n=40)	57.82 ± 5.17 vitamin D sufficient	112.6±6.72	831.7±122.3	BMI: 18.5 - 24.9 (n=40)		

Table 2: Genotypic and allelic distribution of rs2282679 in different vitamin D status groups among CAD patients and Controls. Odds ratios were used to test for the association of the genotype with the disease. The similarity of both distributions suggests that none of the observed genotypes or alleles is found to affect CAD incidence.

rs2282679	Vitamin D status	Controls (n=109)	Patients (n=112)	OR (95% CI)	P-value
	Deficient, N (%)	2 (1.83%)	48 (42.86%)		

TT (Wild type)	Insufficient, N (%)	2 (1.83%)	26 (23.21%)	TT vs GT	0.2513
	Sufficient, N (%)	66 (60.55%)	5 (4.46 %)		
GT (Heterozygous)	Deficient, N (%)	0	11 (9.82%)	0.7179 (0.4125 to 1.285)	
	Insufficient, N (%)	1 (0.92%)	12 (10.71%)		
	Sufficient, N (%)	38 (34.86%)	10 (8.93%)		
GG (Mutant)	Deficient, N (%)	0	0		
	Insufficient, N (%)	0	0		
	Sufficient, N (%)	0	0		
G		39 (17.9%)	33 (14.7%)	1.307	0.3022
T		179 (82.1%)	191 (85.27%)	(0.7888 to 2.211)	

Table 3: Comparison of the association of the observed genotypes of rs2282679 of *GC* gene with circulating 25(OH)D₂, 25(OH)D₃ and total 25(OH)D levels among different study groups. P-values are calculated using unpaired T-test. None of these genotypes had an effect on s-25(OH)D levels except for the wild type (TT genotype) which was significantly associated with lower s-25(OH)D₂ concentrations in the combined subjects, indicated by a p-value of 0.02.

<i>GC</i> (rs2282679)	Genotype	25(OH)D ₃ mean (ng/ml)	p- value	25(OH)D ₂ mean (ng/ml)	p- value	Total 25(OH)D	p- value
	TT: Wild type						
	GT: Heterozygous						
	GG: Mutant						
Patients (n=112)	GT (n=32)	14.99±2.163	0.163	8.184±1.834	0.106	23.11±3.069	0.06
	TT (n=80)	11.77±1.265		5.407±0.784		17.16±1.553	
Controls (n=109)	GT (n=39)	34.35±5.7	0.266	35.21±4.396	0.003	69.55±7.115	0.556
	TT (n=70)	43.42±4.163		19.61±2.27		63.02±5.014	

Total	GT (n=71)	23.38± 3.235	0.5902	19.89±3.271	0.02*	42.23±5.503	0.094
subjects	TT (n=150)	23.64± 2.592		10.73±1.301		34.36±3.493	
(n= 221)							

Table 4: The table shows that the mean s-VDBP level is slightly higher in the Control group than in Patients group. T-test revealed that this small difference isn't statistically significant, p-value = 0.4969.

	VDBP levels (µg/ml)
Patients (n=112)	808.0±65.14
Controls (n=109)	859.8±67.85
p-value	0.4969

Figure Captions:

Figure 1: Comparison of 25OHD3, 25OHD2 and total 25OHD levels among different groups of Controls and Patients. T-test is used to determine the significance. Results are expressed as mean ± SEM and p-values ≤ 0.05 are considered significant.

Figure 2: Compares the mean serum PTH levels among both CAD patients and controls. Unpaired T-test is used to determine its significance. Results are expressed as mean ± SEM and p-values ≤ 0.05 are considered significant.

Figure 3: Comparing the mean s-PTH levels among different vitamin D status groups in controls, patients and total subjects. Vitamin D status is found to be inversely correlated with mean serum PTH levels (P-value=0.005) in the total subjects and vitamin D deficient people have the highest mean s-PTH levels.

Figure 4: Testing for PTH correlation with total 25OHD in the total subjects. A weak inverse correlation existed between both and indicated by spearman correlation coefficient (r) = - 0.3362.

Figure 5: Association of the different genotypes of rs2282679 of *GC* gene with s-25OHD₂ levels among Controls, Patients and total subjects. The wild type (TT genotype) is significantly associated with lower s-25(OH)D₂ concentrations in both the control group and the total subjects, indicated by p-values of 0.003 and 0.02, respectively.

Figure 6: Genotypic distribution of rs2282679 among vitamin D status subgroups in CAD patients and Controls. None of the observed genotypes is found to influence CAD incidence or the total 25OHD levels.

Figure 7: Graphical representation showing that no significant association existed between vitamin D-status and s-VDBP levels among Patients, Controls and Total subjects. One way Anova was used to test for significance.

Figure 8: shows that the different genotypes of rs2282679 of the *GC* gene didn't affect the circulating VDBP levels. P-values were calculated using T-test.

89x57mm (600 x 600 DPI)

130x89mm (300 x 300 DPI)

93x64mm (600 x 600 DPI)

Correlation between PTH and total 25(OH)D in total subjects

50x33mm (300 x 300 DPI)

92x70mm (600 x 600 DPI)

112x74mm (300 x 300 DPI)

119x80mm (300 x 300 DPI)

95x75mm (600 x 600 DPI)