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PRESENCE OF LOW VITAMIN D AND HIGH SERUM LIPOPROTEIN 'A' LEVELS IN CAD: DO THEY IMPART RISK IN SYNERGISM?

Biochemistry

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ABSTRACT

AIMS: Coronary artery disease (CAD) is an inflammatory disorder. Recently low vitamin D and high lipoprotein 'a' (Lp'a') have been linked in causation of coronary artery disease. This study was conducted to see the the combined effect of these two modifiable risk factors i.e. low vitamin D and high Lp'a' in etiology of CAD. **METHODS:** It was a cross sectional study. Triple vessel disease patients (n=31) admitted for bypass surgery were taken as cases and age and gender matched healthy persons were taken as controls (n=30). Serum vitamin D estimation was done by competitive ELISA method. Serum Lp'a' estimation was done by immunoturbidimetric assay. **RESULTS:** when subjects were compared for dual risk factor (Vitamin D deficiency and Hyperlipoproteinemia'a'), it was observed that the odds of having disease were very high (OR=30.00; p=0.0004) than the single risk factor (OR for Hypovitaminosis D = 3.33; p=0.03 and OR for Hyperlipoproteinemia 'a' = 5.00; p=0.004). The Pearson's correlation coefficient for the relationship between serum Vitamin D and Lipoprotein 'a' shows no correlation (r = 0.1019). **CONCLUSION:** Risk of having CAD increases many folds when subject has vitamin D deficiecy as well as high Lp'a'.

KEYWORDS

BACKGROUND

Cardiovascular diseases (CVD) are the leading causes of death in India and around the world. Coronary artery diseases (CAD) constitute the major fraction of CVD.[1] By 2025, it is expected that 7.8 million deaths would occur due to cardiovascular events rising from 5.9 million in 2013.[2] CAD is a multifactorial chronic inflammatory disease and recently, low vitamin D and high lipoprotein 'a' have been identified as risk factor for CAD.

Vitamin D inhibits c-jun-n-terminal kinase (JNK) signaling pathway which result in downregulation of CD36 scavanger receptor on the surface of macrophage resulting in decreased uptake of LDL and reduced foam cell formation.[3-5] Vitamin D also inhibit pro-inflammatory cytokine (IL-6, IL-12, IFN-γ, TNF-α) by regulating MAPK-1 (Mitogen-activated protein kinases) pathway in monocytes and macrophages.[6,7] Proliferation of vascular smooth muscle cell is a key event during plaque formation. Vitamin D inhibits G_1 to S phase progression by down-regulating the expression of F-box protein skp-2 leading to inhibition of cyclin dependent kinase (CDK2).[8] Interaction of vitamin D with vitamin D receptor (VDR) on endothelial cell results in decreased expression of plasminogen activator inhibitor, tissue factor and other potent coagulation factors.[8-10] Hence, role of vitamin D has been found in almost every stage of CAD pathogenesis from endothelial dysfunction to foam cell formation to thrombogenesis and plaque formation.

Lp 'a' has been independently associated with CAD as a risk factor[11-13] but its routine use for assessing cardiovascular risk is still not popular, at least in India. Lp'a' increases the permeability of endothelium by impairing the barrier function of endothelium by causing some rearrangements in actin cytoskeleton which leads to increased deposition of bad cholesterol in the vessel wall.[14] Lp 'a' promotes inflammation in vessels by inducing inflammatory cytokine production (IL-8, TNF-α) and increasing monocyte chemotactic protein (MCP-1) expression. Lp 'a' is seen to act as prothrombotic factor. It increases the production of tissue factor and further augments thrombosis by inhibiting tissue factor pathway inhibitor.[15] Apo 'a' part of Lp 'a' has structural homology with plasminogen but has no fibrinolytic activity. It competes with plasminogen for binding with fibrin thus preventing the plasmin mediated clot lysis and promoting the thrombotic state.[15,16] Along with it Lp 'a' also inhibits the secretion of tissue plasminogen activator (tPA) from endothelial cells and increases the expression plasminogen activator inhibitor (PAI-1).[15,17,18] Apart from its thrombogenic effects Lp 'a' is also associated with smooth muscle cell proliferation and migration by inhibiting TGF-β activation.[19]

Hence it is clearly evident that both vitamin D deficiency and Lp 'a' promotes endothelial inflammation which is at the core of CAD pathogenesis. In this study we have evaluated both vitamin D and Lp 'a' together in CAD patients to assess the risk of Coronary Artery Disease.

MATERIAL AND METHODS

It was a case control study conducted at Vardhman Mahavir medical college and Safdarjung hospital, New Delhi. Thirty one angiographically diagnosed coronary artery disease patients admitted in the Cardio-Thoracic and Vascular Surgery ward for Coronary Artery Bypass Grafting were taken as cases. Thirty healthy individual, matched for age and gender were taken as control. Subjects having acute and chronic renal disorder, liver diseases, thyroid disorder, stroke, myocardial infarction in last 6 months, diabetic ketoacidosis, nonketotic hyperosmolar state were excluded from the study. The study was conducted after clearance from ethical committee of Safdarjung Hospital. Proper informed consent was taken from each subjects before including them into the study. Samples were collected after overnight fasting using all standard procedures. Serum lipoprotein 'a' was estimated on the same day and serum vitamin D was estimated within two weeks. Samples were stored at -20 C. The estimation of serum Vitamin D was performed by competitive ELISA technique. The kit was procured from DLD Diagnostika GMBH. The ELISA kit utilizes a newly designed monoclonal antibody which is equally specific for both forms of Vitamin i.e., Vitamin D_2 and D_3. Serum Lipoprotein 'a' estimation was done by immunoturbidimetric assay with the commercially available Randox system pack kit LP3403 on automated chemistry analyzer Hitachi 902. Statistical analysis was done on SPSS version 21 using appropriate statistical methods. The data are presented as mean ± standard deviation. The difference in the mean base line values of various parameters was analyzed by unpaired t-test or Mann-Whitney test depending upon whether the values were parametric or non-parametric. Odd's ratio were calculated using fisher's exact test. The correlation between two parameters was made using Pearson's correlation. P-value of less than 0.05 was considered significant.

RESULTS

Table 1 shows the comparison of baseline characteristics, serum lipoprotein 'a' and serum vitamin D. Deficiency of Serum vitamin D levels was found in both cases and controls according to Lips P reference range but the mean serum vitamin D level was significantly lower in cases as compared to controls ($p < 0.05$).[20] Serum lipoprotein 'a' levels were found higher in both case and control group (normal levels were considered below 30 mg %). Significantly higher levels of lipoprotein 'a' were observed in cases as compared to controls. When

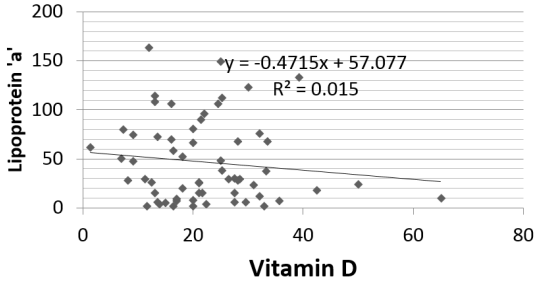
subjects were compared for dual risk factor (Vitamin D deficiency and Hyper-lipoproteinemia'a'), it was observed that the odds of having disease were very high (OR=30.00; CI = 3.16-284.5; p=0.0004) than the single risk factor (OR for Hypovitaminosis D = 3.33; CI = 1.14-9.78; p=0.03 and OR for Hyperlipoproteinemia 'a' = 5.00; CI = 1.67-14.93; p=0.004) (Table 2). No correlation is found between vitamin D and lipoprotein 'a'. (Pearson's correlat-ion coefficient = -0.1019; p-value = 0.33) (Graph-1).

Table 1: Baseline characteristics, Lipid profile, Vitamin D and Lipoprotein 'a' comparison

	Case (n=31)	Control (n=30)	p-value
Age (years)	57.8±7.1	56.5±6.5	0.448
Male (%)	24	21	-
Body Mass Index (kg/m2)	22.4±2.9	23.3±3.6	0.295
Waist to Hip Ratio	0.84±0.1	0.81±0.12	0.307
Diabetes mellitus	22.5%	-	-
Hypertension	70.9%	-	-
Previous myocardial infarction	12.9%	-	-
Statin user	64.5%	-	-
Smoker	25.8%	-	-
Triglyceride	163.87±59.38	145.03±100.55	0.0310*
Total Cholesterol	158.12±53.55	172.63±31.09	0.0541
HDL	33.58±9.66	43.83±15.61	0.0065**
LDL	93.09±31.96	117.5±30.12	0.0043**
Serum Vitamin D (ng/ml)	18.3±8.7	26.1±11.6	0.008
Serum Lipoprotein 'a' (mg/dl)	61.2±44.2	31.4±33.8	0.002

Table 2: Comparison of risk assessment of CAD with Lp 'a', Vit D alone and together

Parameter		Cases	Control	Odd's Ratio	'p'-value
Lipoprotein 'a'	<30 mg/dl	11 (35.4%)	22 (73.3%)	5	0.004
	>30 mg/dl	20 (64.5%)	8 (26.6%)		
Vitamin D	<20 ng/ml	17 (54.8)	8 (26.6%)	3.33	0.037
	>20 ng/ml	14 (45.2%)	22 (73.3%)		
Lp 'a' + VitD	L>30, V<20	12 (38.7%)	1 (3.3%)	30	0.0004
	L<30, V>20	6 (19.3%)	15 (50%)		



Graph1: Correlation between Vitamin D and Lp 'a'

DISCUSSION

The findings in our study show that the Vitamin D levels were significantly lower while serum Lp 'a' levels were significantly higher in CAD patients than the healthy controls. In linear regression analysis, major independent determinant of Lp 'a' are phosphate, SGPT and creatinine while the major independent determinant of vitamin D are BMI, gender and creatinine.

Our findings are in agreement with other studies, showing significantly lower mean value of serum Vitamin D in CAD patients than otherwise healthy individuals.[21] The result of this study showed that there is a close relationship between serum Vitamin D and Coronary Artery Disease.

As we have discussed earlier CAD is basically an inflammatory disorder and Vitamin D has got anti-inflammatory potential and so has got protective role in pathogenesis of CAD. So, lower levels of Vitamin D observed in patients support the evolving theory that Vitamin D deficiency contributes to the development of CAD hence can be considered an emerging risk factor for CAD.

Vitamin D deficiency (levels < 20 ng/ml) was found in 58.4% of Cases in contrast to 26.6% of healthy controls. On applying Fisher's exact test it was found that Vitamin D deficiency increases the susceptibility of having Coronary Artery Disease. (OR=3.33; 95% CI=1.140 9.785; P-value <0.05). These findings suggest that Vitamin D deficiency is significantly higher in CAD patients than the normal healthy individuals.

It further emphasizes the role of Vitamin D in CAD. In our study Vitamin D deficiency was present in subjects with CAD and also in the healthy Controls, which is in agreement with other Indian studies.[21,22] These findings regarding controls, point that Vitamin D deficiency in our country is present in otherwise healthy population. The cause of hypovitaminosis D in a country like India where UV-B radiations are available in plenty might be due to high melanin pigmentation of skin, inadequate sun exposure due to working indoors during the hours when UV-B is available and overclothing. It might also be due to reduced dietary intake of Vitamin D (as availability of fortified food is very uncommon) and injudicious use of sunscreen lotion with higher SPF these days.[23] There might be some genetic factors associated with it preventing the adequate Vitamin D synthesis in Indians like increased 25-OH-D-24-hydroxylase activity, VDR polymorphism, VDBP polymorphism etc.[24-26] Though apparently healthy, they are harbouring potential risk of developing CAD because of low serum vitamin D levels. It is an important area to be focused as hypovitaminosis D can be easily corrected by supplementation and sun exposure.

In our study it was also seen that Lp 'a' levels were significantly higher in Cases as compared to Control. Our findings are similar to other studies.[27,28] These findings suggest that high levels of serum Lp 'a' are associated with CAD which might be due to the pro-atherogenic and pro-thrombogenic effect of Lp 'a'. So, Lp 'a' can be considered as an independent risk factor for CAD.[15-18] Hyperlipoproteinemia 'a' (levels > 30 mg%) was seen in 64.5 % Cases and 26.6% and 36.6% of Controls and Relatives respectively. On applying Fisher's exact test we found that Hyperlipoproteinemia 'a' increases the risk of Coronary Artery Disease in Case group as compared to Control group. Results of our study and other Indian studies suggest that high level of serum Lp 'a' is seen more in CAD patients than the normal healthy controls. These findings further strengthen the theory of pro-atherogenic and pro-thrombogenic effect of Lp 'a'. It was also observed in our study that near one quarter of the healthy subjects were having high lipoprotein 'a' levels, which emphasizes the need for routine use of Lp 'a' estimation for screening in a country like India which is rapidly becoming the capital of CAD.

When results of subjects were analyzed to assess the dual risk of Vitamin D deficiency and Hyperlipoproteinemia 'a' existing together, it was observed that chances of development of CAD are strikingly higher (OR=30.00) than the single risk factor (OR for Hypovitaminosis D = 3.339 and OR for Hyperlipoproteinemia 'a' = 5.00). This means when both the risk factors co-exist, the chances of having CAD increases many fold. These findings suggests that Hypovitaminosis D and Hyperlipoprotein-emia 'a' not only act as an independent risk factor but also act in synergy to pose higher risk when present together. As we have discussed earlier, CAD is an inflammatory disorder. When anti-inflammatory and anti-thrombogenic effect of Vitamin D are lacking and pro-inflammatory and pro-thrombogenic effect of high Lp'a' are present, they may become more pathogenic. The Pearson's correlation coefficient for the relationship between serum Vitamin D and Lipoprotein 'a' shows no correlation (r = 0.1019). These findings suggest that both the risk factor should be screened hand in hand as we suspect they have some synergistic role to play between them.

In nutshell, High Lp 'a' and low Vitamin D are hidden risk factors because they do not have any obvious clinical presentation like other risk factors of CAD viz. obesity, hypertension, diabetes mellitus etc. Both of these may be included into routine screening for coronary artery disease and moreover both of them are modifiable. Hyperlipo-proteinemia can be corrected by Niacin supplementation and Vitamin D deficiency can also be corrected by supplementation and sun exposure.

Since it was a cross sectional study the causal link of vitamin D and lipoprotein 'a' for CAD cannot be established so horizontal studies are required for further evaluation.

CONCLUSION

From the present study it is concluded that vitamin D deficiency and high lipoprotein 'a' levels are more prevalent in patients of Coronary Artery Disease. Low Vitamin D levels and high Lipoprotein'a' levels may be an independent risk factors for CAD. The risk increases many fold when high Lipoprotein'a' and low Vitamin D levels are present together as they might be merging on a common platform to promote inflammation. However to confirm the findings further studies with larger sample size are needed.

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