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Evaluation of serum 25-hydroxyvitamin D levels in calcific rheumatic mitral stenosis— A cross sectional study

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ABSTRACT

Background and aim of the study: Rheumatic mitral stenosis (RMS) is an autoimmune, progressive destructive valve disease occurring as a sequele of streptococcal infection. Epidemiological studies support an association of vitamin D deficiency with initial susceptibility and severity of autoimmune diseases. The aim of the present study was to assess serum level of 25 hydroxyvitamin D in subjects of RMS and assess if any correlation exists with serum levels of vitamin D and severity of disease along with calcification assessed semi-quantitatively by echocardiography by applying Wilkins score.

Method: Fifty five patients of RMS without any calcification of the valves (Group A) assessed by echocardiography along with fifty five patients of RMS with mild to moderately calcified valves (Group B, Wilkins calcium score 1 or 2) and 55 patients with severely calcified valves (Group C, Wilkins calcium score 3 or 4) were enrolled for the study. All subjects underwent clinical, echocardiographic, and biochemical evaluation. The total Wilkins score, Wilkins calcium score along with serum level of 25 hydroxyvitamin D was evaluated in all the patients.

Results: The median serum level of 25 hydroxyvitamin D was significantly lower in Group B (20.4 ng/ml, $p < 0.001$) and group C (11.4 ng/ml, $p < 0.001$) compared to Group A patients (27.9 ng/ml). Similarly serum level of 25 hydroxyvitamin D in Group C patients were significantly less than Group B patients ($p < 0.001$). A significant inverse correlation was identified between serum level of 25 hydroxyvitamin D and total Wilkins score ($r = -0.65$, $p < 0.001$) as well as Wilkins calcium score ($r = -0.69$, $p < 0.001$). But no correlation was identified between 25 hydroxyvitamin D levels and other echocardiographic parameters of RMS.

Conclusion: Our study showed a significantly lower level of 25 hydroxyvitamin D in subjects of RMS with severely damaged and calcified valves as compared to those with less severely damaged non-calcified valves and it correlated with both Wilkins score and Wilkins calcification score. Thus a link may exist between vitamin D deficiency (an immunomodulator) and severity of autoimmune injury on the valves.

1. Introduction

Rheumatic heart disease (RHD), an autoimmune disease is the most common cause of valvular heart disease in developing countries including India. Calcification of the rheumatic-afflicted valves is an important complication that has been reported to occur in 25.2% to 36.4% of patients.[1,2] Though traditionally rheumatic valve calcification was thought to be a passive process, Rajamanann et al.[3] have convincingly shown it to be a regulated inflammatory process associated with expression of osteoblast markers and neoangiogenesis. The valve interstitial cells undergo differentiation into osteoblast like cells which leads to remodeling of the extracellular matrix and biomineralization of the valves. Low level of vitamin D is associated with increased risk of extraskeletal (vascular intimal, valvular and medial artery) calcification. Vitamin D deficiency has been postulated to promote extraskeletal calcification through release of inflammatory cytokines, decrease in calcification inhibitors like fetuin A and matrix GIa protein and inhibition of klotho, whose role is to prevent extraskeletal osteoblastic matrix deposition.[4,5]

In the background of close association of Vitamin D deficiency with pathological valvular calcification, Yavuz et al.[6] in a small interesting study have shown significant deficiency of vitamin D in patients of RHD with calcified mitral valves compared to normal healthy subjects. However it was a small study with 34 subjects and had enrolled only 4 patients with severely calcified valves.

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Epidemiological studies have shown an association of vitamin D deficiency with the severity of autoimmune diseases.[7] Hence we decided to conduct this study with larger number of patients to assess the relationship if any, of vitamin D deficiency (an immunomodulator) with the severity of calcification along with severity of structural damage of the rheumatic afflicted mitral valves, assessed semi-quantitatively by applying the Wilkins score.[8]

2. Methods

2.1. Patients

The study population comprised of 165 symptomatic patients aged ≥ 18 years of age with isolated rheumatic mitral valve stenosis with or without associated mitral regurgitation. Patients with concomitant significant aortic valve disease (moderate to severe aortic stenosis and/or aortic regurgitation) were excluded from the study. Among 165 patients, 55 patients had evidence of severe calcification of the mitral valve (Wilkins calcium score 3 or 4), 55 patients had mild to moderate calcification (Wilkins calcium score 1 or 2) and remaining 55 patients had non calcified mitral valves.

2.2. Sample size calculation

Based on a previous report of significantly decreased level of vitamin D in calcific mitral valve disease by Yavuz et al.[6], using effect size as 0.27, common standard deviation of vitamin D as 0.5, with 80% power and 5% level of significance, the minimum sample size was determined as 138 subjects (46 subjects with severe calcific rheumatic mitral valve, 46 with mild to moderate calcific mitral valve and rest 46 with non-calcific rheumatic mitral valves). Since vitamin D levels have skewed distribution and we planned to apply non-parametric methods to analyze this variable (to retain similar power as ANOVA), the sample size was increased by 15%. Thus a sample size of 55 patients per group, totaling to 165 patients were enrolled for the study.

Patients with chronic liver or kidney disease (serum creatinine >1.5 mg%), endocrine disorders affecting calcium metabolism (hyper/hypoparathyroidism, hyper/hypothyroidism), connective tissue disorders, malignancies, treatment with drugs that could affect serum levels of calcium, phosphorus and albumin, acute rheumatic carditis, infective endocarditis, and acute or chronic systemic infections were excluded from the study. An inability to provide informed consent or comply with the study protocol also caused patients to be excluded from the study.

The study was approved by the ethical committee of our institute and written informed consent was obtained from each patient.

2.3. Study procedure

Demographic variables of patients such as age and gender were recorded. All patients underwent a detailed clinical evaluation to assess their New York Heart Association (NYHA) functional class, severity of valvular disease and to exclude any concomitant conditions such as acute rheumatic fever, infective endocarditis, systemic infections and connective tissue disorders.

Echocardiography was performed in all the subjects. Transthoracic echocardiography (TTE) and transesophageal echocardiography (TEE) in case of suboptimal TTE, was performed using Philips i E 33 instrument. The echocardiography was performed by the same operator in all patients to avoid interobserver variability. In case of doubt regarding the severity of valvular disease or calcification, opinion of a second senior operator not involved in the study was sought and consensus reached. The severity of mitral stenosis with or without associated regurgitation along with severity of associated aortic valve disease if present, was assessed as described previously (following American College of Cardiology/American Heart Association Guidelines).[9,10] The left ventricular dimensions and left atrial dimensions were determined in the parasternal long axis view and left ventricular ejection fraction was calculated using biplane method. The pulmonary artery systolic pressure (PASP) was estimated using tricuspid regurgitation jet applying the simplified Bernoulli equation. The total Wilkins score along with Wilkins calcium score was assessed individually in all subjects to determine the severity of valve damage along with calcification.

Table 1
Baseline characteristics of patients

	Degree of calcification			p-value
Parameters	Group A	Group B	Group C	
Number	55	55	55	
Age in yr*	25 (22–30)	34 (26–42.2)	40 (34–45)	$p < 0.001^a$; $p = 0.034^b$, $p < 0.001^c$
Male	26 (47.3%)	25 (45.5%)	27 (49%)	0.930
Female	29 (52.7%)	30 (54.5%)	28 (51%)	
Associated diseases				
Atrial fibrillation	7 (12.7%)	7 (12.7%)	8 (14.5%)	0.949
NYHA Class				
II	39 (70.9%)	37 (67.2%)	38 (69%)	0.918
III	16 (29%)	18 (32.7%)	17 (30.9%)	
Drug therapy				
Beta blocker	39 (70.9%)	37 (67.2%)	38 (69.0%)	0.918
Calcium channel blocker	16 (29%)	18 (32.7%)	17 (30.9%)	
Digoxin	5 (9%)	6 (10.9%)	5 (9%)	0.933
Diuretics	55 (100%)	55 (100%)	55 (100%)	1.000
Warfarin	7 (12.7%)	7 (12.7%)	8 (14.5%)	0.949

Group A—non-calcified valves, Group B—mild–moderate calcified valves, Group C—severely calcified valves.
Values are in number with percentage or median * with inter quartile range, NS—not significant.
[a]A vs B, [b]B vs C, [c]A vs. C, p-value adjusted for Bonferroni correction

2.4. Laboratory measurements

All biochemical investigations were carried out in a blinded manner. Baseline laboratory tests such as hemogram, renal function test, liver function test, and estimates of serum calcium and phosphorus were conducted in all patients (blood samples were collected form antecubital vein, using 19G needle after overnight fasting) on the same day using standard kits and methods. For the assessment of 25–hydroxyvitamin D (25[OH]D) and parathyroid hormone (PTH), the serum was separated by centrifugation and stored at −70 °C until taken for analysis. Elecsys and Cobas immunoassay analyser (Roche diagnostics GmbH) was used to estimate both 25(OH)D and PTH by electrochemiluminescence immunoassay. 25(OH)D sufficiency, deficiency and severe deficiency were defined as levels ≥30 ng/ml, 10–29 ng/ml, and<10 ng/ml respectively as has been used in other studies.[11]

2.5. Stastical analysis

The data was analyzed by SPSS version 16 statistical software (Chicago, IL). Normally distributed data has been presented as mean (SD) and skewed data as median (interquartile range). One-way ANOVA followed by Tukey's test was applied to compare the mean among the three groups, when homogeneity of variance condition was fulfilled and this condition was tested by the Levene test. Welch test was applied when homogeneity of variance condition was violated and Dunnett T3 post hoc test was applied for multiple comparisons. Kruskal Wallis test was performed to compare the distribution of non normally distributed variables. Multiple comparisons were performed by Mann-Whitney *U* test and p-value was corrected using Bonferroni test. The chi-square/Fisher's exact test was applied to compare the proportion of vitamin D deficiency among the three groups. Pearson correlation was applied to find the correlation between vitamin D with Wilkins calcium score and total Wilkins score. Vitamin D was log transformed to remove right skewness and analysis of variance was applied to adjust the covariate. The adjusted covariate vitamin D has been presented as the geometric mean with 95% confidence interval.

3. Results

Our study population comprised of 3 groups: Group A consisted of patients of rheumatic mitral stenosis with non-calcified valves (Wilkins calcium score 0); Group B of rheumatic mitral stenosis with mild to moderate calcification (Wilkins calcium score 1 or 2) and Group C of rheumatic mitral stenosis with severe calcification (Wilkins calcium score 3 or 4) as assessed by echocardiography.

In our study, the median age of patients of Group A (25 yr) was significantly lower compared to Group B (34 yr, p value <0.001) and Group C (40 yr, p value < 0.001). The median age of Group B patients was also significantly lower compared to Group C patients (p = 0.034 Table 1). There was no significant difference in male to female ratio among the 3 groups. All the patients of our study had severe mitral stenosis with no significant difference in mitral valve area among the three groups as assessed by planimetry or mean gradient across the valve (Table 5). The total Wilkins score assessed by echocardiography was significantly higher in Group C patients (10.96 ± 1.80) compared to Group B (7.07 ± 0.96, p < 0.001) and Group A patients (4.13 ± 0.75, p = 0.001). Similarly, the total Wilkins score in group B patients was significantly higher as compared to Group A patients (p < 0.001, Table 5). There was no significant difference in levels of hemoglobin, blood urea, serum creatinine, ESR, CRP, calcium, phosphorus or alkaline phosphatase (Table 2) across the 3 groups.

The median serum level of 25(OH)D was significantly lower in Group B (20.4 ng/ml, p < 0.001) and Group C (11.4 ng/ml, p < 0.001) patients with calcified mitral valves as compared to Group A patients (27.9 ng/ml) with non-calcified mitral valves (Table 3 and Fig. 1). Similarly serum level of 25(OH)D in patients of Group C was significantly less as compared to patients of Group B (p < 0.001, Table 3). Further in our study, while 20 (36.4%) patients with non-calcified valves had sufficient level of 25(OH)D (≥ 30 ng/ml), only 8 out of 110 patients with calcified valves (7.3%) had sufficient level of 25(OH)D (p< 0.001) (Table 4). All these 8 patients with sufficient level of vitamin D had evidence of mild valvular calcification and none of the patients with severe valvular calcification had sufficient vitamin D levels (Table 4). Similarly while none of the patients with noncalcified valves had evidence of severe Vitamin D deficiency (<10 ng/ml), almost one fifth patients (20%) with calcified valves had severe vitamin D deficiency (p < 0.001 Table 4). So in our study, 92.7% patients with calcified valves had insufficient vitamin D level (72.7% being deficient and 20% severely deficient, Table 4) as compared to 63.6% patients with non calcified valves (Table 4).

Deficiency of vitamin D causes compensatory secretion of PTH to maintain normal level of serum calcium and phosphate.[12,13] In our study, PTH was found to be significantly higher in Group C patients who had the lowest level of 25(OH) D compared to Group A patients (p = 0.004, Table 3). Similarly, the level of PTH in Group B patients was higher than in Group A patients but the difference failed to achieve statistical significance (Table 3).

We found a significant negative correlation between 25(OH)D level and severity of mitral valve calcification as assessed by Wilkins calcium score (r = −0.69 p < 0.001, Fig. 2). Similarly significant negative correlation was found between total Wilkins

Table 2
Comparison of biochemical parameters among the three group of patients

Parameters	Group A	Group B	Group C	p-value
Calcium (mmol/dl)*	1.1 (1.0–1.2)	1.1 (1.0–1.3)	1.2 (1.0–1.3)	0.405
Phosphorus (mmol/dl)*	3.4 (3.1–3.8)	3.6 (3.1–3.8)	3.6 (3.1–4.0)	0.375
Alkaline Phosphatase (IU/L)$	111.6 ± 14.4	106.6 ± 18.4	101.4 ± 12.6	0.102
Total protein (g/dl)*	7.7 ± 0.3	7.5 ± 0.4	7.2 ± 0.6	0.059
Serum albumin (g/dl)*	4.1 ± 0.2	4.1 ± 0.3	4.0 ± 0.5	0.258
Blood urea (mg/dl)*	31 (28–36)	28.5 (24–33.7)	30 (22–34)	0.069
Serum creatinine (mg/dl)*	0.9 (0.6–1.1)	0.8 (0.6–1.0)	0.7 (0.4–1.0)	0.067
Haemoglobin (g/dl) *	11.6 (10.8–13.2)	12.4 (11.1–13.8)	12.6 (11.2–14.1)	0.135
ESR (mm/h)*	20 (16–22)	20 (20–24)	20 (18–22)	0.057
CRP (mg/dl)*	2.0 (1.4–2.6)	1.7 (0.9–2.6)	2.1 (1.6–2.8)	0.125

Group A – non-calcified valves, Group B – mild – moderated calcified valves, Group C – severely calcified valves.
Parameters expressed $as mean ± SD, parameters expressed with * as median with interquartile range.

Table 3
Comparison of Hormone profile among the three groups of patients

				p-value (Bonferroni adjusted)		
Parameter	Group A	Group B	Group C	A vs B	B vs C	A vs C
Serum PTH (pg/ml)	32.8 (26.4–46.2)	38.8 (32.7–48.8)	40.8 (34.6–52.8)	0.548	0.146	0.004
Serum 25 OH Vitamin D (ng/ml)	27.9 (22.4–33.4)	20.4 (14.8–26.8)	11.4 (8.6–16.3)	<0.001	<0.001	<0.001

Group A—non-calcified valves, Group B—mild–moderated calcified valves, Group C—severely calcified valves.
Both parameters expressed as median with interquartile range.
Kruskal Wallis test: $p = 0.004$ (Serum PTH), and $p < 0.001$ (serum vitamin D).

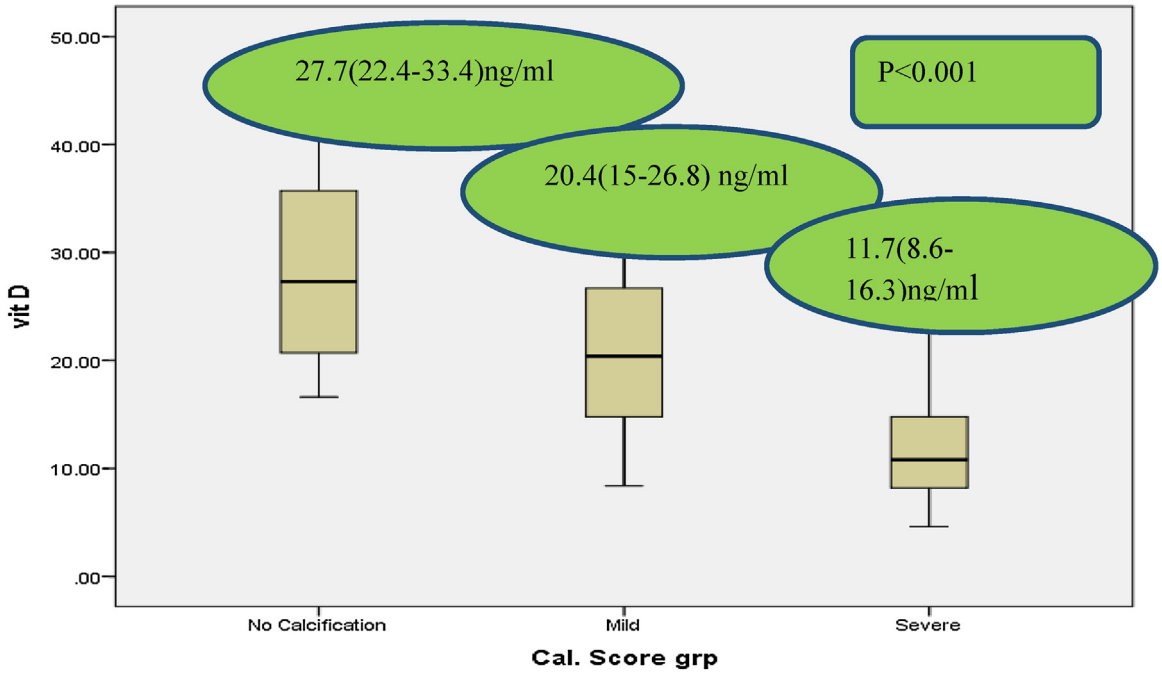

Fig. 1. Distribution of 25 hydroxyvitamin D serum level in patients of rheumatic mitral stenosis without calcification, mild–moderate calcification and severe calcification. The thick lines within the boxes indicate the median, the upper and lower edges of the boxes are the 25th and 75th percentile and the vertical lines extend to the maximum and minimum values.
Correlation between serum vitamin D level and mitral valve calcification score
After applying Pearson correlation test, it was found that there is a very significant negative correlation between serum vitamin D level and mitral valve calcification score ($R = -0.69$, $p < 0.001$)

Table 4
Distribution of Vitamin D level in cases with calcified and non-calcified valves.

	Group A	Group B	Group C	Group B + C	p-value (Bonferroni adjusted) A vs B + C
Severe deficiency (<10 ng/ml)	0 (0%)	2 (3.6%)	20 (36.4%)	22 (20%)	<0.001
Deficiency 10 –<30 ng/ml	35 (63.6%)	45 (81.8%)	35 (63.6%)	80 (72.7%)	0.231
Sufficient (> = 30 ng/ml)	20 (36.4%)	8 (14.5%)	0 (0%)	8 (7.3%)	<0.001

Group A—non-calcified valves, Group B—mild–moderated calcified valves, Group C—severely calcified valves.

score and 25(OH)D ($r = -0.647$ $p < 0.001$, Fig. 3). However, there was no correlation between 25(OH)D level and other echocardiographic parameters of mitral stenosis like mitral valve area, peak gradient or mean gradient across mitral valve.

Though Vitamin D level in group B and group C patients with calcified valves was significantly lower compared to group A patients with non-calcified valves, the age of group B and C patients was also significantly higher compared to group A patients (Table 1). So analysis of co-variance considering serum level of 25 (OH)D (after log transformation) as dependent variable and age as independent variable was applied. The interaction between age in the 3 groups was included to test the homogeneity of slope assumption between vitamin D and age across the 3 groups. The interaction was not significant ($p = 0.159$) revealing that there is no correlation of this assumption. Adjusting the effect of age, adjusted geometric mean value of vitamin D in group B and group C patients with calcified valves was found to be significantly lower than group A patients with non-calcified valves (Table 5).

The baseline echocardiographic parameters are listed in Table 6. All patients of our study had severe mitral stenosis (MVA $\leq 1\ cm^2$). There was no significant difference in valve area assessed by planimetry or mean gradient across mitral valve between the

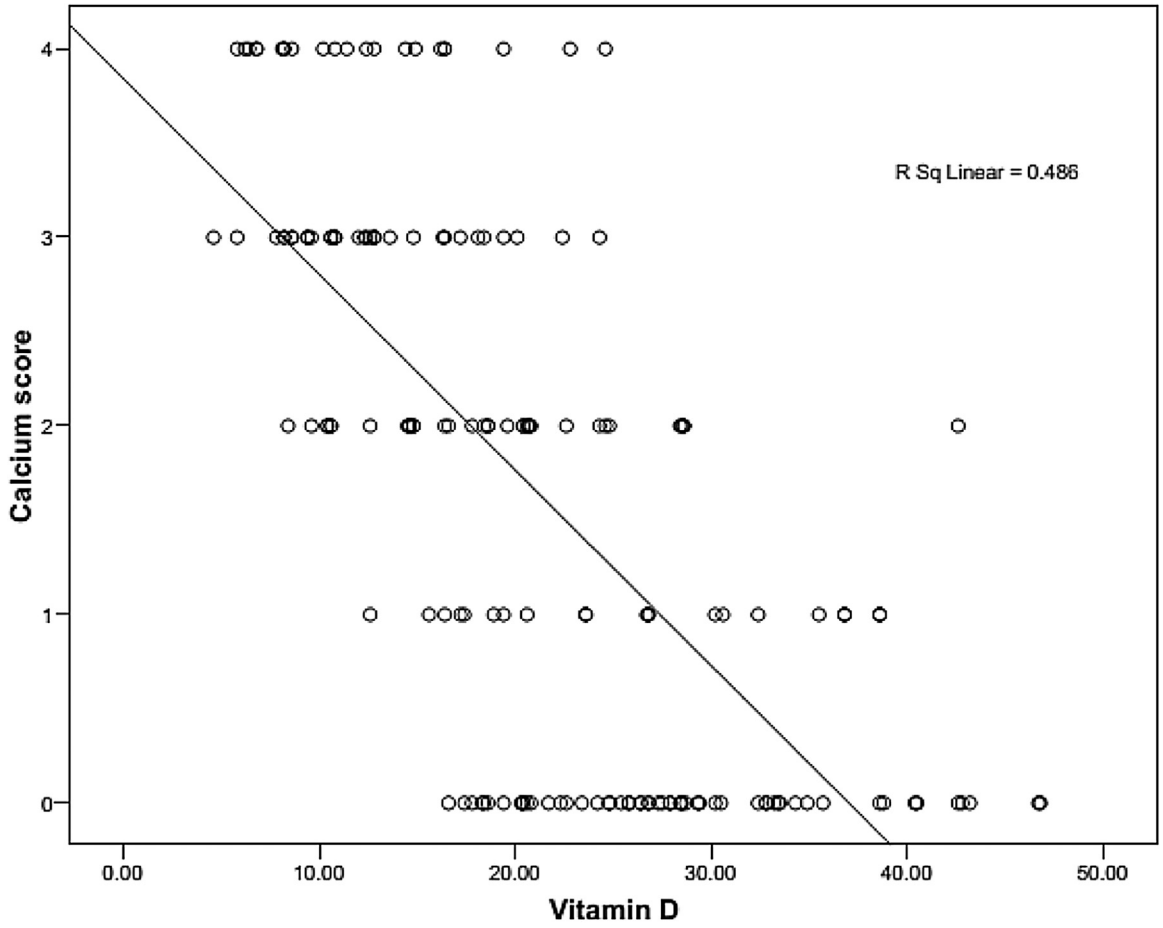

Fig. 2. Scatter Plot: Correlation between serum vitamin D level and calcium score.

three groups (Table 6). Associated moderate to severe mitral regurgitation was present in 18% of patients in group A and C and 20% of patients in Group B. Secondary tricuspid regurgitation was present in all patients across all the groups. The pulmonary artery systolic pressure (PASP) and LV ejection fraction (EF) were similar in all the three groups (Table 6).

4. Discussion

Our study has shown a strong inverse correlation between mitral valve calcification severity assessed echocardiographically by Wilkins score and serum level of 25(OH)D (Fig. 2). A similar strong inverse correlation was also found between total Wilkins score of the valve and serum 25(OH)D level.

Though the Wilkins score was introduced to assess the degree of deformity of the valve apparatus and predict outcome of balloon mitral valvuloplasty, it can also be used to assess the severity of structural deformity as a sequele of immunological damage inflicted on the valve apparatus by the disease process. In our study, both severity of calcification as well as structural deformity of the mitral valve showed a strong inverse correlation with serum level of 25(OH)D.

RHD is an autoimmune progressive, destructive valvular disorder whereby, molecular mimicry between cardiac tissue proteins and streptococcal antigens like M protein leads to valve damage mediated by streptococcal antigen primed auto-reactive CD_4^+ effector T cells.[14] In all autoimmune diseases, it is the extent of imbalance between pro and anti-inflammatory cells that determine the severity of injury on the target organ. In RHD, there is not only over reactivity of effector CD_4^+ T cells but also deficiency of anti-inflammatory CD_4^+ T cells known as regulatory T cells, recently reported by us.[15]

Extensive research over last 25 years has identified CD_4^+ T effector cells to be the predominant cells infiltrating the valves[16–19] and we[20] along with others[21] have shown that the degree of infiltration by mono-nuclear cells is proportional to the severity of calcification. Hence in our study the total Wilkins score was significantly higher in patients with calcified valves compared to non-calcified valves. These infiltrating CD_4^+ T cells inflict tissue injury by differentiating into different types of helper cells like Th1 helper cells[22,23] which secrete cytokines like tumor necrosis factor-alpha (TNF- α), and γ interferon or recently reported Th17 cells[24,25] secreting cytokines like interleukin (IL) 17 and IL 22. TNF- α has also been shown to promote calcification by inducing the valve interstitial cells to undergo differentiation into osteoblasts.[26,27]

On the other hand, vitamin D has been shown to have number of effects on T cells that can modulate the T cell mediated Immune response.[28] Vitamin D is reported to exert its action on T cells through vitamin D receptors (VDR) identified on CD_4^+ T cells.[29] Vitamin D has been reported to induce development of anti-inflammatory regulatory T cells[30] (which is deficient in patients with RHD) and inhibit proliferation of effector CD_4^+ T cells.[31–33] Vitamin D also inhibits production of inflammatory cytokines like TNF-α and γ interferon produced by Th1 helper T cells and IL-17 by Th17 helper T cells.[31,34,35] Apart from this, vitamin D deficiency has been reported to cause decrease in systemic levels of calcification

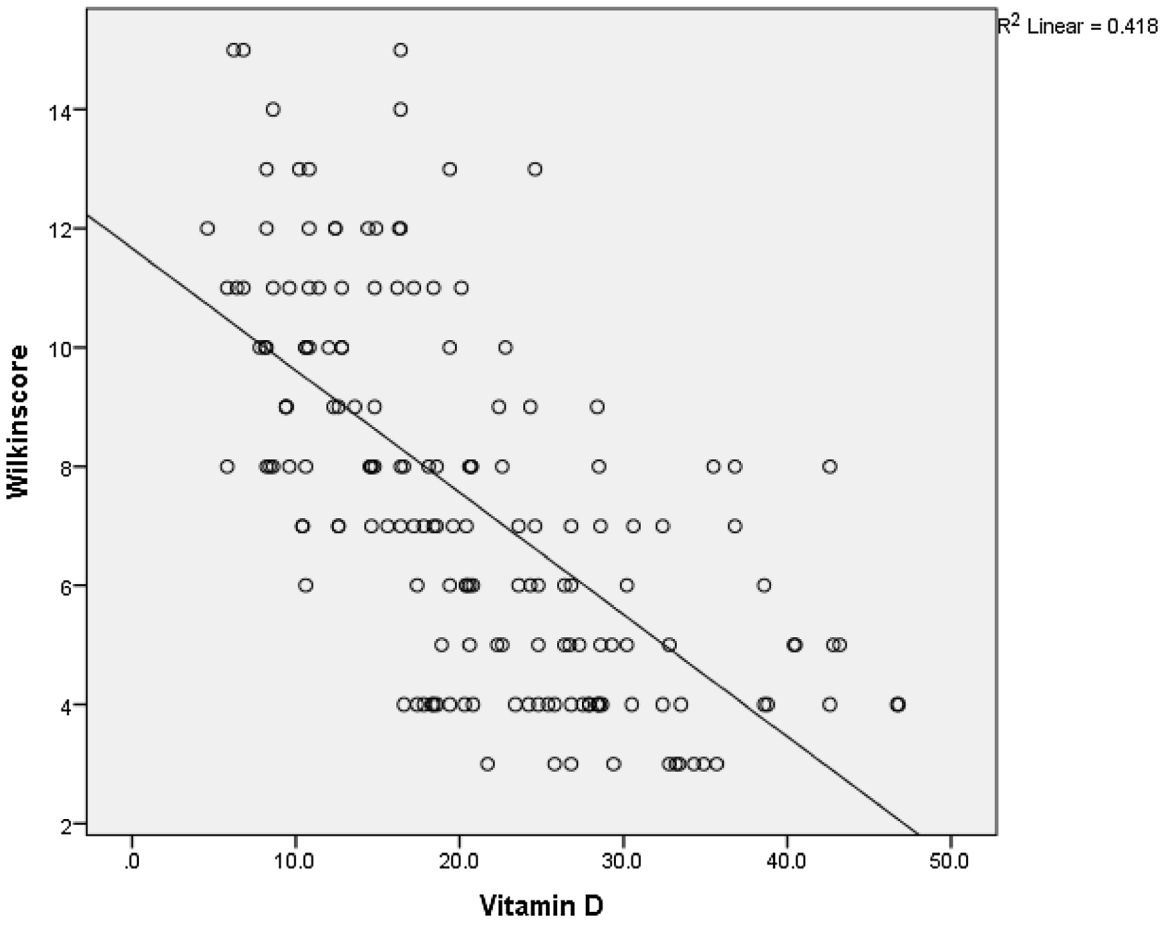

Fig. 3. Scatter Plot: Correlation between serum vitamin D and Wilkins score.

Table 5
Age adjusted vitamin D level comparison in studied patients.

				(p-value (Bonferroni adjusted)		
	Group A	Group B	Group C	Group A vs B	Group B vs C	Group A vs C
Serum 25 OH Vitamin D (ng/ml)	27.61 (24.88–30.62)	19.85 (18.05–21.82)	11.63 (10.50–12.88)	<0.001	<0.001	<0.001

Group A—non-calcified valves, Group B—mild–moderated calcified valves, Group C—severely calcified valves.
Values are presented as geometric mean with 95% confidence interval.
[F-value = 61.59, df (2161), p = 0.000; <0.001].

Table 6
Echocardiographic parameters of the three group of patients.

Parameters	Group A	Group B	Group C	p-value
Mitral valve area (cm^2)				
Planimetry	0.78 ± 0.11	0.76 ± 0.11	0.75 ± 0.11	0.388
Pressure half time	0.80 ± 0.08	0.78 ± 0.09	0.76 ± 0.10	[a]p = 0.342; [b]p = 0.288; [c]p = 0.011
PG across MV	28.49 ± 2.82	29.11 ± 4.51	30.56 ± 5.27	[a]p = 0.734; [b]p = 0.185; [c]p = 0.034
MG across MV	16.82 ± 2.37	16.87 ± 2.78	17.62 ± 3.45	0.273
Associated MR (mod-severe)	10 (18.1%)	11 (20%)	10 (18.1%)	0.961
Associated mild AS	6 (10.9%)	5 (9.09%)	6 (10.9%)	0.937
Associated Mild AR	8 (14.5%)	6 (10.9%)	7 (12.7%)	0.849
Secondary TR (n)	55 (100%)	55 (100%)	55 (100%)	1.00
LVEF (%)	60	59.6	59.8	
PASP (mm Hg)	51.10 ± 13.5	50.5 ± 10.8	50.1 ± 12.9	0.914
Total Wilkins score	4.13 ± 0.75	7.07 ± 0.96	10.96 ± 1.80	[a]p < 0.001; [b]p < 0.001; [c]p < 0.001
Wilkins calcium Score	0	1.67 ± 0.60	3.42 ± 0.45	p < 0.001

Values are mean ± SD, AR—aortic regurgitation. AS—aortic stenosis, MR—mitral regurgitation, MV—mitral valve, MG—mean gradient, PG—peak Gradient, PASP—pulmonary artery systolic pressure, TR—tricuspid regurgitation, LVEF—left ventricular ejection fraction, NS—not significant.
[a] A vs B, [b]B vs C, [c]A vs. C, P-value adjusted for Bonferroni correction

inhibitors like fetuin A (which is already decreased in patients of RHD)[20] and matrix GI protien.[36]

Thus from the various anti-inflammatory effects of vitamin D on T cells, we can infer that significant deficiency of vitamin D in our studied subjects with calcified valves compared to non-calcified valves may be one of the principle factor responsible for exaggerated immune response and greater valvular damage in these subset of patients compared to those with non-calcified valves. Apart from extent of deficiency of vitamin D (promoting an inflammatory procalcific milieu), duration of exposure to this harmful milieu is also a major determinant of severity of structural damage and calcification. Hence in our study, age of patients with severely calcified valves was significantly higher than those with mild to moderately calcified valves, which in turn was also significantly higher than those with noncalcified valves. The numerous beneficial effects of vitamin D on T cells (increased activity of regulatory T cells and decreased activity of effector T cells) is also evident from our study which shows a strong inverse correlation of serum level of vitamin D with total Wilkins score as well as Wilkins calcium score.

As there is no definite therapy available till date to arrest or alter the progressive valvular damage in subjects of RHD, correction of vitamin D deficiency (which was present in 70–90% of our study population) may help to attenuate or arrest the progressive immunological tissue injury inflicted on the valves as has been shown in animal studies in other Th1/Th17 mediated auto-immune diseases like inflammatory bowel disease, multiple sclerosis and Type 1 diabetes.[37–40]

5. Limitation of the study

As our study was a cross-sectional study, causality could not be determined but a significant negative correlation has been demonstrated between serum vitamin D level and severity of structural mitral valve damage along with calcification. Future studies with longitudinal design is required to understand whether optimizing vitamin D level in patients of RHD has potential as a disease modifying intervention.

6. Conclusion

Our study has shown a significantly lower level of 25(OH)D in subjects of RMS with severely damaged and calcified valves compared to those with less severely damaged non-calcified valves and it correlated with both total Wilkins score and Wilkins calcification score. Thus a link may exist between vitamin D deficiency (an immunomodulator) and severity of autoimmune injury on the valves.

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