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Vitamin D Levels and its Correlation with the Severity of Mitral Valve Insufficiency in Rheumatic Heart Disease Pediatric Patients; A Case Control Study from Egypt

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Abstract:

Introduction: Acute rheumatic fever (ARF) is an autoimmune response to B hemolytic streptococcal pharyngitis infection. (1) Rheumatic heart disease (RHD), the permanent injury to the cardiac valves caused by ARF, is a major cause of morbidity and mortality in developing countries all over the globe. Vitamin D level has played a crucial role in the severity of mitral valve regurgitation because of its immunomodulatory effects. (2) **Aim of the study:** The study aims to assess the levels of vitamin D in pediatric RHD patients with mild to severe mitral valve regurgitation. **Methods:** Fifty adolescents with confirmed to have RHD and fifty matched healthy controls diagnosed by 2-Dimensional Transthoracic Echocardiography were recruited from Rheumatic Heart Disease Clinic at Cairo University Specialized, (EGYPT). Serum 25(OH)D concentrations were measured using dried blood spots and anthropometric measures were recorded to evaluate nutritional status. Logistic regression analysis was done to identify an association between vitamin D deficiency and the severity of Mitral regurgitation in RHD. **Results:** Prospective case control cohort study, recruited 50 pediatric patients from Rheumatic Heart Disease Clinic at Cairo University Specialized. The patients were prescribed long-acting penicillin (LAP) for RHD management. The mean age of RHD patients was 12.6 $\pm$ 2.51 years with a 2:1 female to male ratio (62% versus 38%; respectively). There was an inverse weak correlation between vitamin D serum level and the degree of regurgitation; however, the result wasn't statistically significant. Vitamin D levels showed a statistically significant association with patients who were compliant to LAP. All laboratory measures showed no statistically significant differences between trivial to mild and moderate to severe mitral regurg cases except for calcium. **Conclusion:** Vitamin D can play a significant role in predicting severity of mitral valve regurgitation. Future studies are required in Egypt to further predict the other nutritional factors and demographic characteristics in pediatric patients with variable severity of RHD. **Keywords:** Rheumatic Heart Disease-Mitral Insufficiency-Vitamin D- Children

1. Introduction

Acute rheumatic fever (ARF) is a disease caused by the autoimmune response to pharyngitis due to Group A beta hemolytic streptococci (GAS) infection. Rheumatic heart disease (RHD), the long-term damage to the cardiac valves brought on by ARF, is a significant source of morbidity and mortality in developing nations all over the world. It can be brought on by a one, severe episode of the sickness or by recurrent attacks of the illness (3). Following GABHS infection of the pharynx, both B and T cells trigger antibodies in response to the infection, followed by T cell activation (mostly of CD4+ cells). Innate immune system components phagocytose microorganisms and carry antigen to T cells. By using particular antibodies and T cells through molecular imitation, the host response to GAS in susceptible individuals causes autoimmune reactions against host organs; including the heart, joints, brain, and skin (4). The primary cause of rheumatic carditis is an antibody-mediated immunological response to GAS, followed by cellular infiltration of the endocardium and valve tissues, leading to further damage and eventually ends in RHD (4).

Upon the injury of endothelial cells (ECs), the bone marrow releases endothelial progenitor cells (EPCs) into the circulation, which restore the injured blood vessel wall (2). Vitamin D endorses the process of EPCs release from the bone marrow to the blood vessels by affecting metalloproteinase (MMP) and guanosine triphosphate (GTP) activity, and help in EPC adhesion to the injured endothelium (5). Furthermore, since vitamin D controls vascular endothelial growth factor expression, this can provide an explanation for the link between hypovitaminosis D, endothelial damage and RHD (6)

Consequently, many studies have revealed a significant risk between the decrease in vitamin D levels and elevated risk of developing ARF (6) Moreover, a correlation between vitamin D deficiency as an immunomodulatory and severity of autoimmune injury on the valves in rheumatic mitral stenosis (RMS) patients (7).

Although the etiology is known, the exact pathophysiology of RHD development is still not understood. The persistence and worsening of valvular lesions in RHD are thought to be related to autoreactive cells that persist in the circulation (8) only some strains of GAS are rheumatogenic and people have different susceptibility to developing ARF, however the cause of increased susceptibility of a host isn't fully understood. (9) The grave impact that malnutrition has on both the innate and acquired immune system is generally recognized (10).

Hypovitaminosis D in specific is linked to increased susceptibility to infection as GAS pharyngitis in addition to an increased risk of autoimmune diseases (11). Links have been found between ARF and rheumatic mitral stenosis, Since the regulation of expression of vascular endothelial growth factor is related to 25(OH)D, this would provide an explanation to the link between hypovitaminosis D and endothelial dysfunction, and subsequently ARF and RHD (12)

Globally, the incidence of RHD is 3.4 cases per 100,000 population in developed countries escalating to more than 1000 cases per 100,000 in developing countries (13). In Egypt, one study revealed that the prevalence of vitamin D deficiency in healthy adolescents was as high as 99% (14)

Accordingly, this study aimed to assess the level of serum biomarkers, particularly vitamin D and the associated degree of rheumatic mitral valve insufficiency among pediatric patients, no previous studies have researched this group of adolescent age.

Subjects and methods:

Prospective cohort case control study was conducted at Cairo University hospital for children - Cairo-Egypt in the rheumatic heart disease clinic. Fifty patients with the inclusion criteria visiting the clinic for follow up and were prescribed long-acting penicillin from January 2022 to September 2022 were recruited. The patients were classified into either trivial/ mild regurge or moderate/ severe regurge groups.

Ethical considerations:

Legal guardian written informed consent was obtained for each included patient. The study protocol confirmed confidentially according to the declaration of Helsinki [15]. Every participant was explained the purpose of the study and assured the confidentiality. The research ethics committee (REC) at Faculty of Medicine, October 6 University reviewed and approved the research under approval number: PMC-Me-2202012.

Inclusion criteria:

1. Patients aged 10 to 18 years.
2. Confirmed rheumatic heart disease (mitral insufficiency) by 2-D Echocardiography in accordance with the World Heart Federation echocardiographic guidelines.
3. On Follow up schedule every 3-4 weeks giving secondary long-acting penicillin prophylaxis.
4. Legal guardians agreed to follow up at the rheumatic heart clinic.

Exclusion criteria

Patients with ANY of the following criteria were excluded;

1. Age less than 5 years
2. Patients on follow up and regular prophylaxis with long-acting penicillin intramuscular (IM) for arthritis, not carditis or have valve lesion other than mitral regurgitation.
3. Rheumatic heart disease patients who suffer from other associated disease that affects the immune system or diseases that could interfere with Vitamin D metabolism; as recent hospital admission for more than one week, chronic kidney disease, thyroid disease and active tuberculosis or receiving steroids

Control Group

Controls were selected by medical history taking and echocardiography among any patients, relatives or acquaintants, matched gender and age

Methods:

The following was done for all subjects included in this study:

1. Thorough History Taking:

- a) Demographic data including; age, gender, first presentation, age of diagnosis, compliance to long acting penicillin and complaint of joint pain at the timing of examination.
- b) Clinical examination; including anthropometric measures, weight and height, general examination; including heart failure and systemic disease interfering with vitamin D metabolism.
- c) Cardiac examination including inspection and palpation: to exclude ventricular enlargement, pulmonary hypertension, auscultation: for mitral regurge or other valve disorders.

2. Laboratory Tests:**Biochemical measurements**

Aseptic venipuncture was done to withdraw 6 ml of blood divided into two red-topped serum separator tubes (3 ml) each.

I- Routine investigations:

First the serum tube; the blood was left to clot for 15 minutes. Then it was centrifuged within 1h from withdrawal. Blood was centrifuged at 3000 rpm (1000 X g) for 10 minutes. Serum was harvested after centrifugation and was kept into 2 aliquots (1.5 ml micro-centrifuge tube each) in -20°C till calcium profile assesment, including total calcium and ionized calcium.

II-Specific investigations:

The second serum aliquot was used to asses serum 25 hydroxy vitamin D level by commercially available ELISA technique. Assay of serum 25 hydroxy vitamin D used *sandwich kit*; Bioassay technology laboratory (1008 Junjiang Inter. Bldg, 228 Ningguo Rd, Yangpu Dist 200090, Shanghai, China).

3. Two-Dimensional Trans thoracic Imaging Echocardiography:

Echocardiography was performed in all the subjects. Philips *i E 33 instrument manufactured in Norway* was used for performing the transthoracic echo. The same operator performed the echocardiography in most patients to eliminate inter-observer variability.

Diagnosis of Rheumatic heart disease was based on two-dimensional Echo Doppler and color flow Doppler; including:

M-Mode Echocardiography:

Measurement of left ventricular end-diastolic volume (LVED), left ventricular end-systolic volume (LVES), left atrium (LA) diameter, right ventricle (RV) diameter, left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS). Complete 2-D study with Doppler for cardiac valves when Mitral regurge is suspected; *the first step* was to document the criteria of pathological regurge.

Echocardiographic criteria include [16];

- a) Regurgitant jet more than 1 mm in length.
- b) A regurgitant jet in at least 2 planes.
- c) A mosaic colored jet > 2.5 m/sec
- d) Jet that persists through systole (mitral valve) or through diastole (aortic valve) .

The second step was to document the severity of regurgitation and was categorized according to Color flow Doppler mapping into a six- point scale as follows (16):

- 0: Nil, physiological or trivial regurgitant get <1 cm, narrow, small, short duration, early systolic at mitral or early diastolic at aortic
- 0+: very mild regurgitant jet (Trivial) more than 1 cm, wider, localized immediately above or below the valve, throughout the systole at the mitral valve or throughout the diastole at the aortic.
- 1+: Mild regurgitant jet
- 2+: Moderate, longer and at a wider area
- 3+: Moderately severe jet, reaching the entire left atrium in mitral regurge or left ventricle in Aortic regurge
- 4+: Severe regurgitant jet diffusely into enlarged left atrium with systolic backward flow into the pulmonary veins

Associated cardiac anomalies were looked for.

Statistical Methods:

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count and percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and or Mann-Whitney tests. For comparing categorical data, Chi square (X²) test was performed. Fisher exact test was used instead when the expected frequency is less than 5. Correlations between quantitative variables were done using Spearman correlation coefficient.

P-values less than 0.05 was considered as statistically significant.

Sample size calculation: all the patients who applied the inclusion criteria during the period from January 2022 to September 2022 were recruited

Results:

Table 1: Demographic distribution and anthropometric measurements of the studied cases with RHD:

Degree of MV regurge			Trivial / mild MR	Mod/ severe MR	Total	P value
Gender	Female	N	24	7	31	>0.999 ⁽¹⁾
		%	63.2%	58.3%	62.0%	
	Male	N	14	5	19	
		%	36.8%	41.7%	38.0%	
Age	Mean± SD		12.67±2.65	12.09±2.02	12.6±2.51	0.302 ⁽²⁾
	Min- Max		10-18	10-16	10-18	
Height	Mean± SD		147.15±12.94	146.64±12.04	147.02±12.6	0.622 ⁽²⁾
	Min- Max		120-170	128-171	120-171	
Weight	Mean± SD		41.62±13.12	38.45±12.7	41.08±12.96	0.409 ⁽²⁾
	Min- Max		23-70	25-60	23-70	

(1) Fisher exact test (2) Independent T test SD standard deviation. *:Statistically significant at $P \leq 0.05$.

Table 1: presents the demographic distribution of the studied cases diagnosed with RHD, age ranged 10-18 years with a mean 12.6+/-2.5 year, females constituted a larger percentage to males (62% versus 38%; respectively, ratio 2:1). Their anthropometric measurements were; their weight ranged from 23-70 Kg with a mean of 41.08+/-12.9 kg while Height ranged from 120-171cm and a mean of 147.02+/-12.6 cm, showing a good nutritional status. No statistical difference between the two Groups (Trivial/Mild MR and the Moderate to Severe MR group).

Polyarthritis, Chorea and silent carditis were the commonest presentation in trivial+/- mild mitral valve (MV) regurge cases; However, carditis and heart failure were the commonest presentation in moderate+/- severe MV regurge group ($P=0.008$), ($P=0.003$) respectively (table 2).

2-D Echocardiography findings revealed, statistical significant thickened mitral valve , left ventricular end Diastolic dimension ($P < 0.001$) ($P=0.024$) respectively among moderate +/- severe MV regurge cases compared to the trivial +/- mild regurge MV group. Also, associated pathology was significantly associated with the moderate+/- severe MV regurge group compared to trivial+/- mild MV regurge group ($P=0.012$) (table 2).

Table 2: Clinical presentation of the studied patients to the RHD Clinic:

Degree of Mitral valve regurge			Trivial/ mild	Mod/ severe	Total	P value
Rheumatic fever presentation	Polyarthrititis	N	22	6	28	0.008^{*(1)}
		%	57.9%	50.0%	56.0%	
	Silent carditis	N	13	1	14	
		%	34.2%	8.3%	28.0%	
	Manifest carditis	N	2	5	7	
		%	5.3%	41.7%	14.0%	
	Chorea	N	1	0	1	
		%	2.6%	0.0%	2.0%	
Leaflets	Normal	N	28	0	28	<0.001^{*(2)}
		%	73.7%	0.0%	56.0%	
	Thickened	N	10	12	22	
		%	26.3%	100.0%	44.0%	
Ventricular dimension enlargement	No	N	36	8	44	0.024^{*(2)}
		%	94.7%	66.7%	88.0%	
	Yes	N	2	4	6	
		%	5.3%	33.3%	12.0%	
Heart failure	No	N	36	8	44	0.003^{*(2)}
		%	100.0%	66.7%	91.7%	
	Yes	N	0	4	4	
		%	0.0%	33.3%	8.3%	
Joint pain	No	N	16	4	20	0.729 ⁽²⁾
		%	47.1%	36.4%	44.4%	
	Yes	N	18	7	25	
		%	52.9%	63.6%	55.6%	
Associated pathology	No	N	29	4	33	0.012^{*(3)}
		%	76.3%	33.3%	66.0%	
	Yes	N	9	8	17	
		%	23.7%	66.7%	34.0%	
Compliance to LAP	No	N	22	6	28	0.473 ⁽³⁾
		%	57.9%	50.0%	56.0%	
	Yes	N	16	6	22	
		%	42.1%	50.0%	44.0%	

(1) Montecarlo test (2) Fisher exact test (3) Chi square test *: Statistically significant at $P \leq 0.05$, LAP: long acting penicillin.

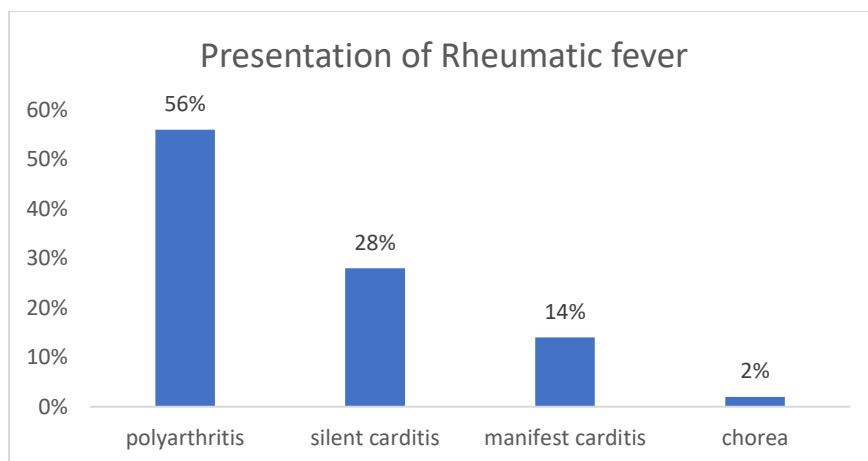


Figure (1). The different first presentations of Rheumatic heart disease

Among the whole cases, RHD first presentations was polyarthrititis in 56 % of the cases where as silent carditis was found in 28% of the cases that discovered by ECHO, 14% have manifest carditis and 2% have chorea (figure 1).

Table 3 and figure 2 reveal associated pathology of patent ductus arteriosis (PDA), atrial septal defect (ASD), chorea (one case each) mild aortic regurgitation, (5 cases) among Trivial+/- mild MV regurge. Moderate+/- severe MV regurge cases showed associated Hyperthyroidism, mild to moderate tricuspid regurge (5 cases), dilated left atrium, mild aortic regurge (one case) each showing statistical significance when both groups compared (P value= 0.021)

Table (3): Associated pathology with mitral insufficiency

		Trivial mild	&	Moderate severe	&	Total	P value
PDA amplatzer	N	1		0		1	0.021*
	%	12.5%		0.0%		6.3%	
Hyperthyroidism	N	0		1		1	
	%	0.0%		12.5%		6.3%	
ASD	N	1		0		1	
	%	12.5%		0.0%		6.3%	
Mild tricuspid regurge	N	0		4		4	
	%	0.0%		50.0%		25.0%	
Mild aortic regurge	N	5		1		6	
	%	62.5%		12.5%		37.5%	
Dilated left atrium	N	0		1		1	
	%	0.0%		12.5%		6.3%	
Moderate tricuspid regurge	N	0		1		1	
	%	0.0%		12.5%		6.3%	
Chorea and on safinaz	N	1		0		1	
	%	12.5%		0.0%		6.3%	

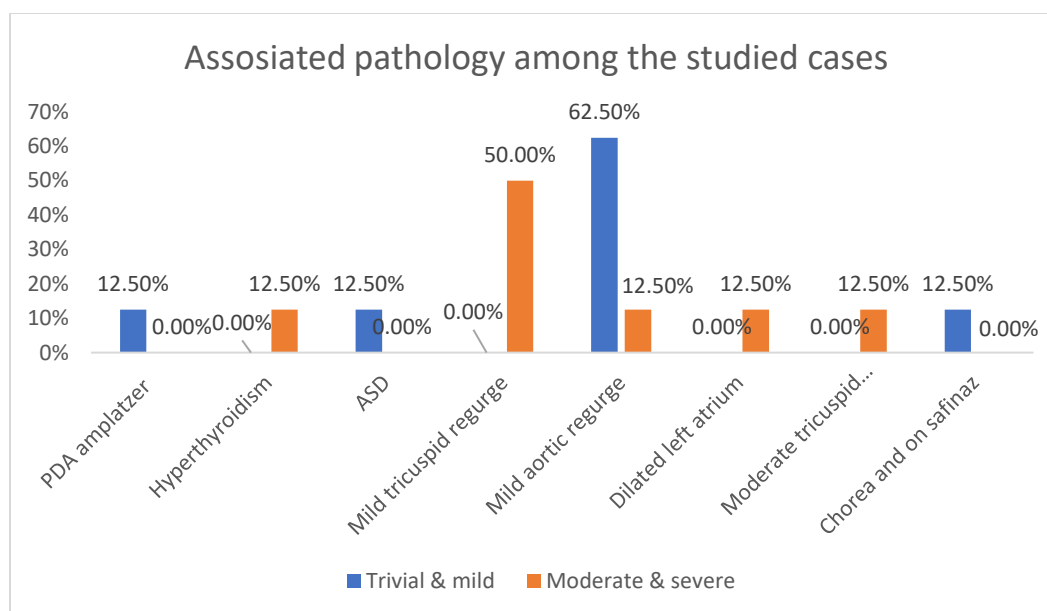


Figure (2): Associated pathology among the studied cases:

Table 4: Laboratory investigations among the cases:

			Trivial & mild	Moderate & severe	Total	P value
CBC	Normal	N	30	9	39	>0.999 ⁽¹⁾
		%	78.9%	75.0%	78.0%	
	Anemia	N	8	3	11	
		%	21.1%	25.0%	22.0%	
Vit D	Median (IQR)		16.75(5.2)	16(6.2)	16(5.2)	0.369 ⁽²⁾
	Min- Max		10.7-55	12.6-39	10.7-55	
ASOT	Median (IQR)		342.5(426)	400(450)	361(430)	0.532 ⁽²⁾
	Min- Max		98-1200	185-800	98-1200	
ESR	Median (IQR)		16.5(22)	11(24)	16(22)	0.927 ⁽²⁾
	Min- Max		4-105	4-80	4-105	
CRP	Median (IQR)		4(2)	5(17)	4(8.5)	0.152 ⁽²⁾
	Min- Max		3-55	3-48	3-55	
Calcium	Mean± SD		8.42±0.74	7.9±0.57	8.29±0.73	0.041 ^{*(3)}
	Min- Max		7.3-10.4	7-8.7	7-10.4	
Ionized Calcium	Mean± SD		1.02±0.09	0.95±0.11	0.99±0.09	0.298 ⁽³⁾
	Min- Max		0.89-1.25	0.88—1.04	0.88-1.25	
Albumin	Mean± SD		4.78±0.36	4.83±0.37	4.5±0.38	0.721 ⁽³⁾
	Min- Max		3.8-5.5	4.1-5.4	3.8-5.5	

ASOT: antistreptolysin O titre; ESR: erythrocytes sedimentation rate; CRP: C-reactive protein; IQR Interquartile range; SD standard deviation. (1) Chi square test (2) Mann Whitney U test (3) Independent T test. *: Statistically significant at $P \leq 0.05$.

According to Table 4 All laboratory measures showed no statistically significant differences among trivial+/- mild MV regurg group compared to moderate+/- severe MV regurg cases

except for serum calcium level, where calcium concentration among Trivial+/- mild MV regurge cases was statistically higher than that in moderate+/- severe MV regurge cases ($P=0.041$).

Furthermore, 25 hydroxy vitamin D, ionized calcium, albumin and erythrocyte sedimentation rate (ESR) showed no statistical significance in trivial+/-mild MV regurge cases compared to moderate+/- severe MV regurge cases (all $P > 0.05$). Also, Haemoglobin, c-reactive protein (CRP) and antistreptolysin O titre (ASOT) showed no statistical significance between the two groups (all $P > 0.05$).

Vitamin D levels showed a statistically significant higher serum level among patients who were complaint on long-acting penicillin (LAP) treatment, ($p < 0.001$) (Table 5)

Table 5: Comparing serum laboratory test in compliant and non-compliant cases.

M		Compliance to LAP		Total	P value
		No	Yes		
Vitamin D	Median (IQR)	14.2(2.8)	19.15(16.6)	16.65(6.1)	<0.001*
	Min- Max	10.7-19.5	14.2-55	10.7-55	

Mann Whitney U test *: Statistically significant at $P \leq 0.05$

Vitamin D level was significantly lower in RHD patients with the complaint of joint pains (Table 6, figure 3), and was significantly inversely proportional to the complaint of joint pains ($p < 0.001$).

Table (6) and Figure (3): Comparing vitamin D levels in patients with and without joint pain

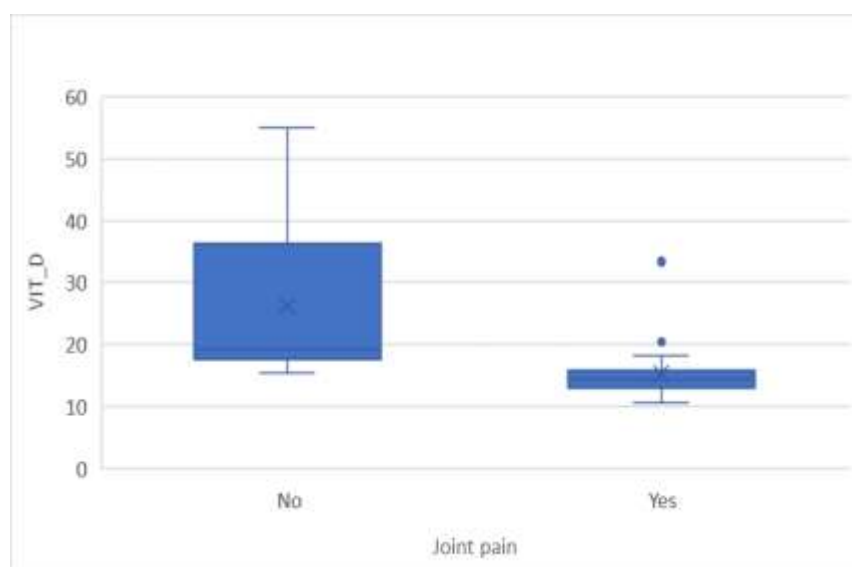


Table (6): vitamin D levels in patients with and without joint pain

	Joint pain	P value
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		No	Yes	
Vit D	Median (IQR)	19.3(18.7)	14.4(2.8)	<0.001*
	Min- Max	15.4-55	10.7-33.4	

Mann Whitney U test *: Statistically significant at $P \leq 0.05$

There was an inverse weak correlation between vitamin D serum level and the degree of regurgitation; however, the result wasn't statistically significant ($P=0.489$) (figure 4).

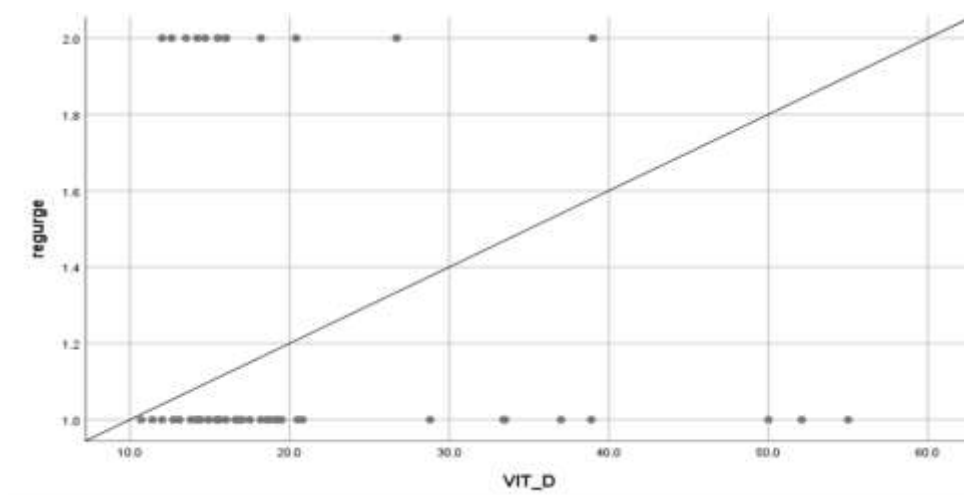


Figure 4: Correlation between 25 hydroxy Vit D level and degree of Mitral valve regurge:

Discussion:

Acute and chronic RHD is the most predominant acquired heart disease in paediatrics. Although RHD is rarely seen in developed countries, it still represents a challenge in developing countries with serious consequent of morbidity and mortality from heart disease⁽¹⁷⁾. The disease is basically limited to poor and low socioeconomic populations and almost all of those patients are present with heart failure [18].

Our study results demonstrated, female predominance than males (62% versus 38%; respectively, ratio 2:1) and the mean age of presentation was 12.6+/-2.5 years. Similar local studies revealed the same findings with a predominance of female gender among RHD patients as well as a mean age of presentation 13 years (19).

According to American heart association (AHA), major diagnostic measures for Rheumatic fever are carditis, arthritis, Chorea, erythema marginatum, and subcutaneous nodules. In contrast, less frequent measures include arthralgia, hyperpyrexia, elevated ESR, and/or elevated CRP. Polyarthralgia and monoarthritis are considered prognostic factors for moderate to severe risk patients. In moderate to high-risk group, monoarthralgia is defined as a minor characteristic [20]. This aligns with our study findings where polyarthritis, Chorea and silent carditis were common in trivial+/- mild MV regurge groups however, manifested carditis (41.7%) was the commonest presentation among moderate+/-severe MV regurge group. Similarly, Al-sekait Saudi Arabia, reported RHD showed frequent carditis at presentation ranging from 58% to 65% of cases (21).

Laboratory biomarkers in the present study revealed an insignificant higher mean value of ESR in trivial+/- mild MR cases compared to moderate +/-severe MR cases. Yet, anemia, CRP and ASOT were higher in moderate +/- severe MR cases ($P > 0.05$) and were statistically insignificant.

Similarly, a study by Zainab et al., 2020 has demonstrated a significant association between blood levels of ASOT and CRP with carditis (22), and another study by Klimpke et al., 2023 demonstrated an association between anemia and RHD. (23) Moreover, Yoshioka et al., 2023 reported albumin was found associated with different stages of heart diseases (24) and Liu et al., 2014 found that calcium level was found associated with rheumatic heart disease. (25)

Heart failure, thickened leaflets and ventricular dimension enlargement were common among the moderate +/- severe MR cases in our study ($P < 0.05$). Although the exact mechanism is not known, Al-Khalifa et al., 2008 revealed that heart failure, thickened leaflets and ventricular dimension enlargement were correlated to worsening in RHD symptoms and deterioration of existing valve lesions. (17)

Additionally, our study revealed that joint pain was more among moderate +/- severe MV regurgitation group compared to trivial+/- mild MV regurgitation group but the results were not statistically significant ($P > 0.05$). This comes in the line with AHA guideline where polyarthralgia and monoarthritis were prognostic factors for moderate to severe risk patients [20].

The current study revealed an insignificant inverse weak correlation between 25 hydroxy vitamin D serum level and the degree of mitral valve regurgitation. In the same line, Onan et al 2017 demonstrated no significant difference among 25 hydroxy vitamin D levels of patients with mild, moderate, or severe carditis however, patients with carditis were associated with lower 25 hydroxy vitamin D levels compared to those without carditis including controls ($p=0.054$) (2)

Similarly, a study conducted by Yusuf et al., 2017 comparing 55 patients with rheumatic mitral stenosis without calcification of the valves to mild to moderately calcified valves and severely calcified valves, revealed significantly lower levels of 25 hydroxy vitamin D in both groups with calcifications of the valves compared to those without calcification (7)

In this study, 25 hydroxy vitamin D levels showed a statistically significant association with patients who were compliant to LAP. This aligns with the results of Ali et al., 2019 study where patients who were compliant to LAP had higher levels of 25 hydroxy vitamin D in comparison to the non-compliant. (19)

Although scarce studies have investigated the association between vitamin D levels and LAP compliance, vitamin D metabolites showed crucial role in both innate and adaptive immune responses, causing a reduction in infection with bacteria and viruses. Furthermore, 25 hydroxy vitamin D is an effective stimulator of the innate antimicrobial response (26). This can justify the results revealed in our study where compliant patients to LAP had higher 25 hydroxy vitamin D levels and with less serious disease stages (i.e. trivial+/- mild cases group).

There was an inverse weak correlation between vitamin D serum level and the degree of regurgitation; however, the result wasn't statistically insignificant ($P=0.489$).

Conclusion:

This study may highlight the crucial role of vitamin D in predicting severity of mitral valve regurgitation. Future large-scale studies are required to demonstrate the effect of the nutritional status of pediatric patients with RHD on the progression of severity of mitral valve regurgitation.

Declarations:

Consent for publication:

All the authors hereby that they revised the manuscript and agreed on the content.

Availability of data and material

All data are available upon request from the corresponding authors.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions:

All authors have equally contributed in conducting this research.

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