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VITAMIN D STATUS IN CHILDREN WITH JUVENILE IDIOPATHIC ARTHRITIS IN ALEXANDRIA UNIVERSITY CHILDREN'S HOSPITAL

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INTRODUCTION

Juvenile idiopathic arthritis (JIA) is the most incessant systemic connective tissue disease occurring at developmental age.[1] It denotes a group of clinically discernible categories that share chronic, childhood-onset arthritis of unknown cause as a integrate characteristics. The exact etiologies of JIA are not well detected and the pathogeneses is still unclear but are likely multifactorial. Conflicts among JIA epidemiologic studies might count for recognized prevalence differences, actual differences might occur as a consequence of genetic, ethnic, environmental, and lifestyle impacts.[2]

Vitamin D status is potentially commanded by the similar factors as JIA; vitamin D receptor genotype, ethnically related skin tone and clothes, environmental differences in exposure to ultraviolet B radiation relating to the housing conditions, season, and vitamin D nutritional intake are factors that modulate serum vitamin D levels. For this reasons special awareness had been given to the involvement of vitamin D in good health and well-being. Vitamin D is incriminated in the pathogenesis of autoimmune diseases including, as examples, multiple sclerosis, type 1 diabetes, Crohn's disease, and asthma because of its notable role as in inflammation and immune mediation.[8-11]

The vitamin D receptors (VDR) is widely designated in the cells of the immune system, including T cells and dendritic cells, implying its important role in immune regulation.[3-6] When bonded to the active vitamin D ligand, the VDR acts as a transcription factor, regulating transcription of vitamin D-responsive genes. Thus, the VDR moderates vitamin D biological effects.[7]

It was also documented that vitamin D influenced directly and indirectly the proliferation and function of T-cells that play the pivotal share in the pathogenesis of JIA. Stimulation of T CD4+/CD25-cells in the presence of 1,25(OH)2D constrained the production of proinflammatory cytokines including IFN-γ (interferon γ), IL-17 (interleukin 17) and IL-21, and influenced the high expression of IL-10 and IL-4 production, also increase in the CTLA-4 and FoxP3 proteins, characteristic of regulatory cells.[7,11] Absence of inhibition of TH17 response in vitamin D deficiency likely contribute to its augmented immune reaction in this case.

Vitamin D may have a part in modulating JIA disease activity and it is familiar to be great importunate in osteoporosis, repeated falls and associated fractures, which are usually present in JIA. The anti‑inflammatory, immunomodulatory, and antiproliferative, effects of Vitamin D may have a valuable therapeutic role.[12] The relationship between the severity of JIA and levels of Vitamin D is a issue of tremendous concern.

Thereupon; this study was undertaken to compare the of Vitamin D serum levels in the healthy children and JIA children attending the rheumatology clinic at Alexandria University Children Hospital and to study the relation if present between these serum levels of vitamin D and disease activity.

MATERIALS AND METHODS

This cross -sectional study was conducted on 45 patients with diagnosis of JIA attending the rheumatology clinic in Alexandria University Children's Hospital. 15 children of matched age and sex with no musculoskeletal complain were taken as control group.

All children with JIA were subjected to through history taking assessing type of JIA according to the to the International League of Associations for Rheumatology (ILAR).[13]

Disease severity was assessed according to the value of DAS28[14] score as follows:

- Remission: DAS28 ≤2.6
- Low disease activity: 2.6 <DAS28 ≤3.2
- Moderate disease Activity: 3.2 <DAS28 ≤5.1
- High disease Activity: DAS28 >5.1.

Inclusion criteria

Both males and females are under the age of 16 years having JIA according to the European League Against Rheumatism were enrolled in this study.

Exclusion criteria

Patients with malnutrition, hepatic and renal dysfunction, hyperparathyroidism, hyperthyroidism, diabetes mellitus, and patients on Vitamin D supplementation in the past 6 months or on medications that can affect bone and vitamin D metabolism (anticonvulsants, diuretics, and thyroxin) were excluded from this study.

Laboratory methods

For all patients and controls: blood samples were collected in the morning after the patients had had an overnight fasting period, which served to minimize diurnal effects. The blood samples were evacuated into 3 types of vacutainer tubes;

- EDTA tubes for complete blood count (CBC)
- Citrated tubes for erythrocyte sedimentation rate (ESR)
- Plain tubes which were used to obtain serum by allowing the blood to clot at room temperature for 15 – 30 minutes, then centrifugation at 3400 rpm for 15 minutes.
- For every subject, CBC and ESR were measured during the same day of samples collection, while serum was separated, aliquotted, and frozen at -70°C to be used later on.
- Serum samples were used for measurement of the following parameters:

25(OH)D, using electro-chemiluminescence binding assay (ECLIA) on Cobas 411 (Competitive protein binding assay). From Roche, US.[15]

- PTH, using electro-chemiluminescence binding assay (ECLIA) on Cobas 411 (Competitive protein binding assay). From Roche, US.[16]
- Total calcium, using colorimetric method. (Biolabo reagent, France).[17]
- Phosphorus, using colorimetric method. (Biolabo reagent, France).[18]
- Alkaline Phosphatase, using colorimetric method. (Biosystems reagent, Spain).[19]
- Magnesium, using colorimetric method. (Biolabo reagent, France).[20]

All continuous variables are reported as mean ± standard deviations (SD).

Vitamin D status was considered to be deficient if serum level < than 10 ng/ml, insufficient from 10-30ng/ml and sufficient from30-70ng/ml.[21]

The study was approved by the ethics committees of Alexandria Faculty of Medicine. Informed consent was obtained from parents or guardians of all participating children.

Statistical Analysis

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version **21.0 IBM Corp., Chicago, USA, 2013.** Descriptive statistics were done for quantitative data as minimum& maximum of the range as well as mean ± SD (standard deviation) for quantitative parametric data, while it was done for qualitative data as number and percentage.

Inferential analyses for independent variables were done using Chi square test for differences between proportions and student t-test for continuous variables, for more than two group ANOVA test was used, Person correlation coefficient was used to find the correlation between each two variables.

The level of significance was taken at P value less than 0.05 is highly statistically significant, otherwise is non-significant.

RESULTS

There was no significant statistical difference between the demographic data of children with JIA and the controlling group regarding age and sex. **Table (1).**

The scope of the data discussed includes: the descriptive parameters of the JIA cases, regarding the age of onset of the disease (with a mean of 6.19±3.04), and the duration of the disease (with a mean of 2.72±2.53). The polyarticular presentation was the most common category, representing 21 (46.7%) patients. The rheumatoid factor was positive in 3 cases out of 21 polyarticular category (14.0%). 15(33.3%) patients were classified with the oligoarticular presentation, 6 (20%) had the systemic onset presentation, and no patients were classified as enthesitis nor considered undifferentiated. Anti-nuclear antibodies (dense fine speckled pattern) was positive in 4 cases out of 15 oligoarticular category (26.0%). The number of tender joints ranged from 0.0-8.0with a mean of 1.60±2.04. The number of swollen joints had a mean of 1.49±1.99. The disease activity score (DAS) was high in 20.0% of the cases, moderate in 40.0% of the cases, and low in 40.0% of the cases; **Table (2).**

On comparing the JIA patients to the control group regarding laboratory findings, a significant difference was found in ESR in JIA patients compared to the control group. The total white blood cells count (WBCs) and platelets show a significant difference in JIA cases and the control group. As for the serum calcium, phosphorus, magnesium, alkaline phosphatase and parathormone levels, no statistically significant difference was found between the two groups. A statistically significant low serum concentration of vitamin D was found in the JIA patients compared to the controlling group (mean: 16.1±6.1, 20.6±6.2 respectively, $p < 0.013$). The vitamin D status in the control group was 1 child (3.3%) deficient, 18 children (60%) insufficient, 11 children (36%) sufficient, while in the JIA patients, the status was 12

patient (26.7%) deficient, 33 patient (73.3%) insufficient, and none of them had a sufficient level. These differences in vit D status were statistically significant (p <0.001) **Table(3).**

Different categories of JIA patients were evaluated for the mean level of vitamin D. We noticed that all cases with systemic onset JIA had the lowest mean level compared to polyarticular and oligoarticular categories. The mean levels were 16.82±4.39, 18.27±3.87 and 22.06±3.75 respectively and the difference was statistically significant between the 3 categories (p=0.016).Vitamin D status followed the following pattern: in JIA categories (Oligoarticular, Polyarticular and systemic onset), 13 patients (39.4%), 18 patients (54.5%) and 2 patients (6.1%) respectively had insufficiency, while 2 patients (16.7%), 3 patients (25%) and 7 patients (58.3%) respectively were deficient; the differences in the previous data according to vitamin D status were statistically significant (p=0.0026)**. Table (4) Fig (1).**

The severity of the disease using DAS showed a significant negative correlation with vitamin D levels. **Table (4) Fig (2).**

Regarding the disease activity score (DAS) correlation with serum levels of calcium, phosphorus, magnesium, alkaline phosphatase, and parathormone; it was found that there was a positive correlation between DAS and both parathormone and alkaline phosphatase levels (p=0.041 and 0.036 respectively) **Table (5).**

The mean of vitamin D serum level correlated negatively with both the disease duration and the number of tender joints. **Figure (3,4)**

We tested the quality of life of JIA cases by using the JAFS score (Juvenile arthritis functionality scale) as well as using VAS (visual analogue score) for well-being and disease activity and both correlated negatively to the mean level of vitamin D serum. **Figure (5,6)**

Table (1): Demographic data of patients with juvenile idiopathic arthritis (JIA) and control group.

	Cases		Control group		P
	Number "n=45"	Percent	Number "n=30"	Percent	
Sex					
Male	18	40.0	11	36.7	0.215
Female	27	60.0	19	63.3	
Age					
<6 years	11	24.4	6	20.0	
6-12	20	44.4	7	23.3	0.107
>12 years	14	31.1	7	23.3	
Range	3-16		3-14		
Mean ±S.D.	8.87±3.62		7.98±2.98		0.099
Median	8.00		8.0		

Table (2): Descriptive parameters of the JIA patients.

	Cases	
	Number "n=45"	Percent
Age of onset		
Range	1.0-13.0	
Mean ±S.D.	6.19±3.04	
Median	6.00	
Duration of disease		
Range	1 m – 10.0 y	
Mean ±S.D.	2.72±2.53	
Median	2.5	
Type of onset of JIA form		
Oligoarticular	15	33.3
Polyarticular	21	46.7
Systemic	9	20.0
Activity of the disease(DAS)		
High disease Activity	9	20.0
Moderate disease Activity	18	40.0
Low disease activity	18	40.0
Number of tender joints		
Range	0.0-8.0	
Mean±S.D.	1.60±2.04	
Median	1.6	
Number of swollen joints		

Range Mean ±S.D. Median	0.0-8.0 1.49±1.99 1.5	
RF Positive Negative	3 12	14.0 66.0
Antinuclear Antibody Positive Negative	4 17	26.0 74.0

Table (3): Laboratory findings in JIA patients and Control group.

	Patients "n=45"		**Control "n=30"**		**p**
ESR 1st hr Range Mean±S.D.	5-70 24.8±16.4		5-16 12.3±3.65		0.013*
ESR 2nd hr Range Mean±S.D.	13-104 48.5±24.0		11-28 18.2±4.21		0.005*
CRP Range Mean±S.D.	0.01-160 17.7±40.4		0.00-5.0 2.72±1.35		0.001*
Hb Range Mean ±S.D.	7.3-14.1 11.5±1.4		8.5-14.2 12.1±1.65		0.125
WBC Range Mean ±S.D.	4.1-25.9 10.6±5.4		4.5-6.5 5.32±1.25		0.003*
Platelets Range Mean ±S.D.	125-714 370.1±137.3		150-450 203.1±87.0		0.011*
Calcium Range Mean ±S.D.	8.8-10.5 9.4±0.4		9.5-11.0 10.2±0.68		0.120
Phosphorus Range Mean±S.D.	4.4-6.2 5.2±0.5		4.2-6.5 5.32±0.64		0.254
ALK. Phosphatase Range Mean ±S.D.	122-343 235.1±65.4		118-352 241.3±52.6		0.236
Magnesium Range Mean ±S.D.	1.7-2.56 2.0±0.2		1.72-2.6 2.21±0.24		0.365
Parathormone Range Mean ±S.D.	11-56 28.3±11.6		14.5-52.0 29.2±10.6		0.421
Vitamin D status Deficiency Insufficiency Sufficiency	12 33 0	26.7% 73.3% 0.0%	1 18 11	3.3% 60.0% 36.7%	0.0035*
Range Mean ±S.D.	8.9-28.0 16.1±6.1		9.0-42.0 20.6±6.2		0.013*

Table (4): Relation between vitamin D status, disease activity score and JIA subtypes.

	Mean Vit. D ±S.D.	Vitamin D status				Total	P
		Deficiency		Insufficiency			
		No.	%	No.	%		
Activity of the disease (DIS) High disease Activity	16.96±4.25	8	66.7	1	3.0	9	0.0019*
Moderate disease Activity	19.84±3.92	4	33.3	14	42.4	18	
Low disease activity	20.83±4.28	0	0.0	18	54.5	18	
P value comparing the mean	0.033*						
Type of onset of JIA form							
Oligoarticular	22.06±3.75	2	16.7	13	39.4	15	0.0026*
Polyarticular	18.27±3.87	3	25.0	18	54.5	21	
Systemic	16.82±4.39	7	58.3	2	6.1	9	
P value comparing the mean	0.016*						

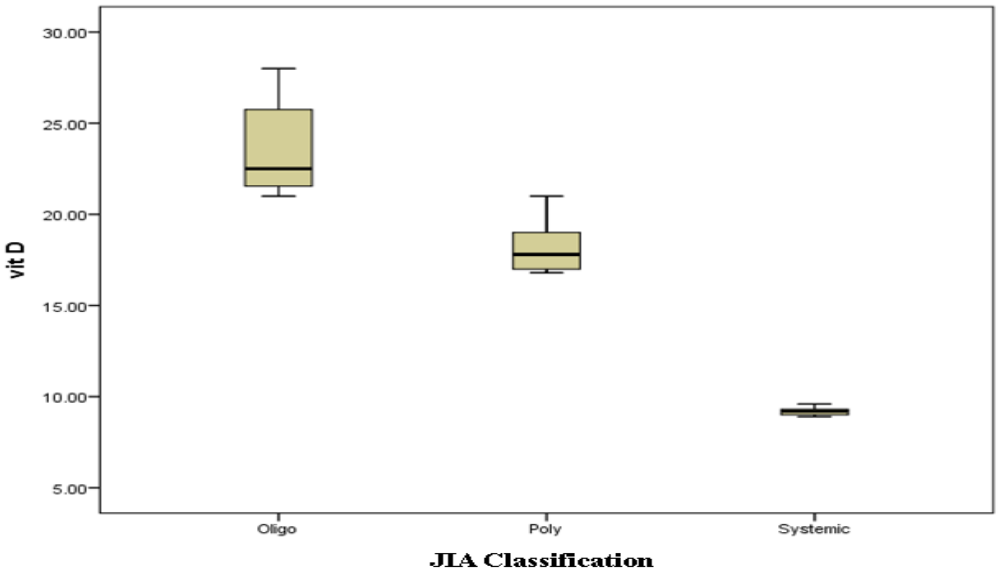

Fig. (1): Relation between JIA categories and the mean serum vitamin D level.

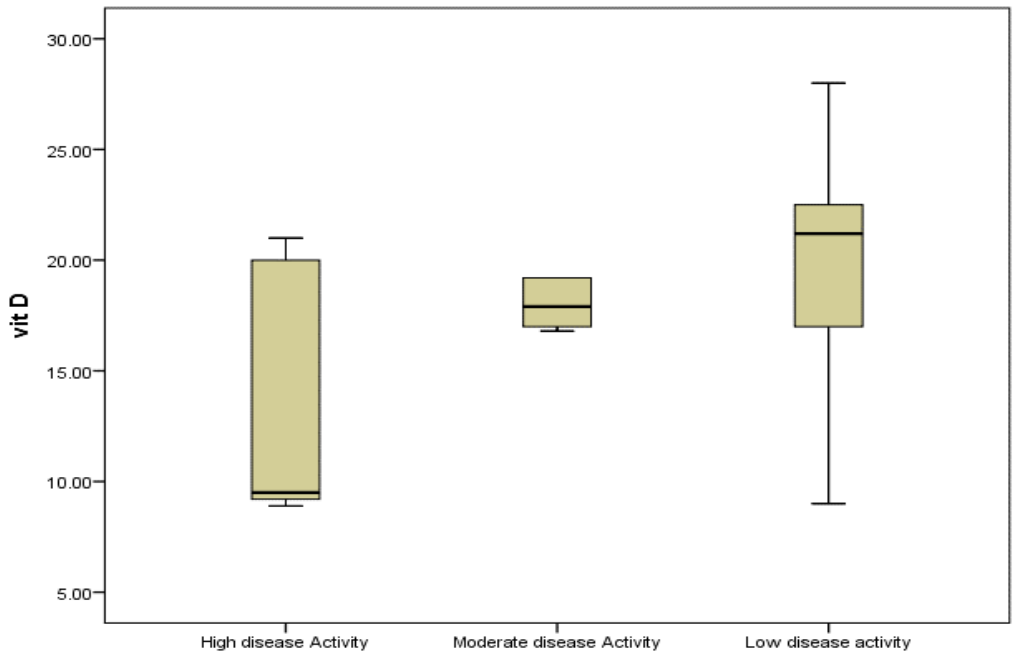

Fig. (2): Relation between disease activity score and the mean serum vitamin D level.

Table (5): Relation between disease activity score and Calcium, Phosphorus, Magnesium, alkaline phosphates and Parathormone levels.

	Low	Moderate	High	P
Calcium	9.31±0.46	9.43±0.37	9.59±0.53	0.322
phosphorus	5.04±0.44	5.14±0.58	5.48±0.52	0.265
Magnesium	1.91±0.23	1.97±0.24	2.04±0.27	0.068
Alkaline phosphates	225.25±53.43	230.22±60.57	244.94±76.76	0.036*
Parathormone	27.37±11.62	28.01±11.80	30.78±11.90	0.041*

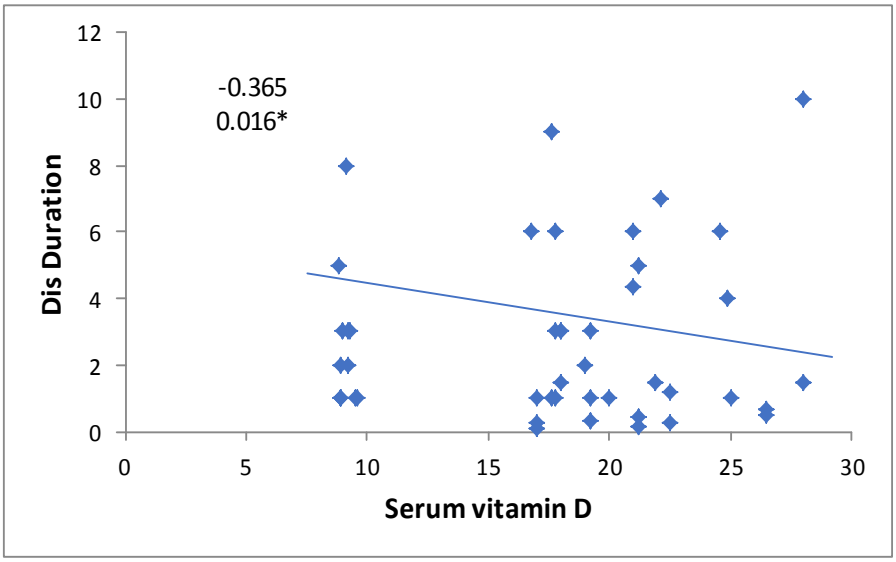

Figure (3): Correlation between vitamin D level and disease duration.

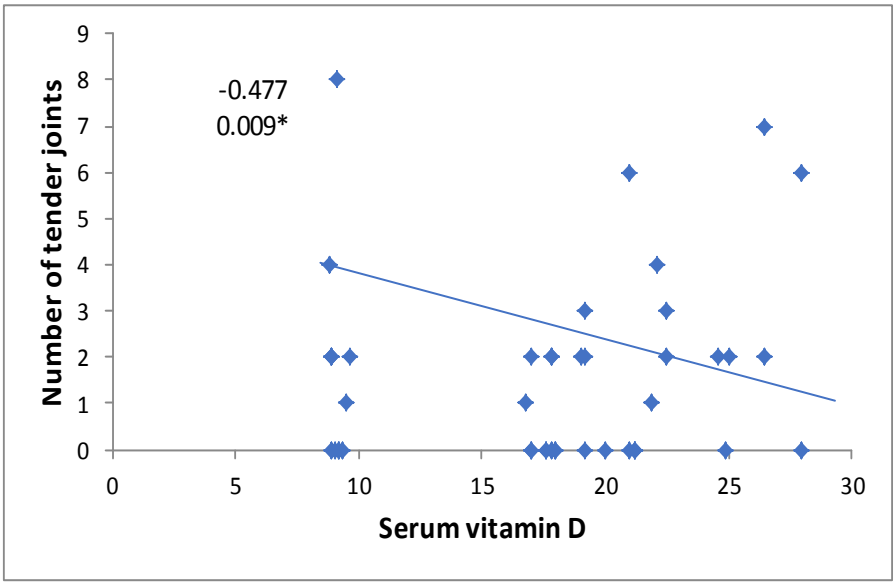

Figure (4): Correlation between vitamin D level and number of tender joints.

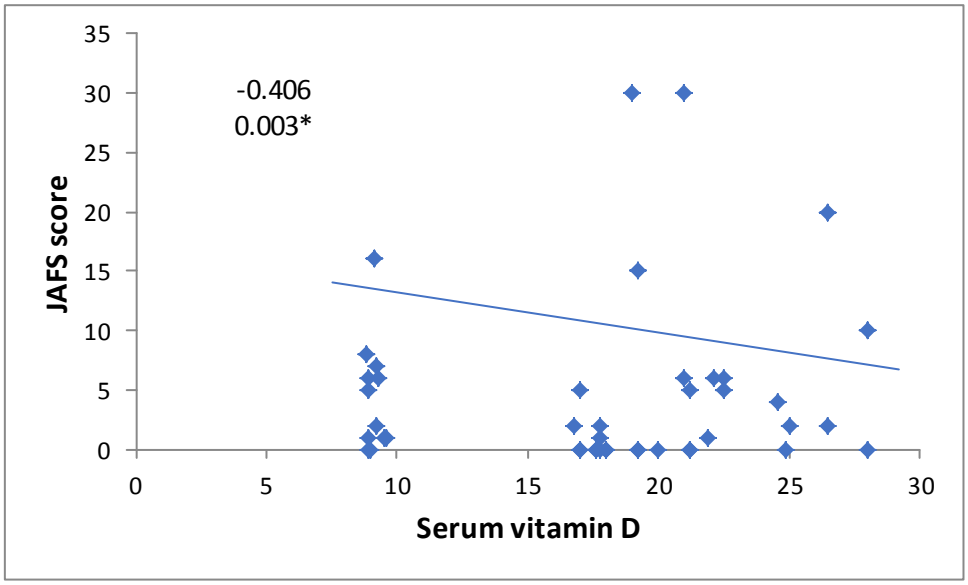

Figure (5): Correlation between vitamin D level and JAFS score.

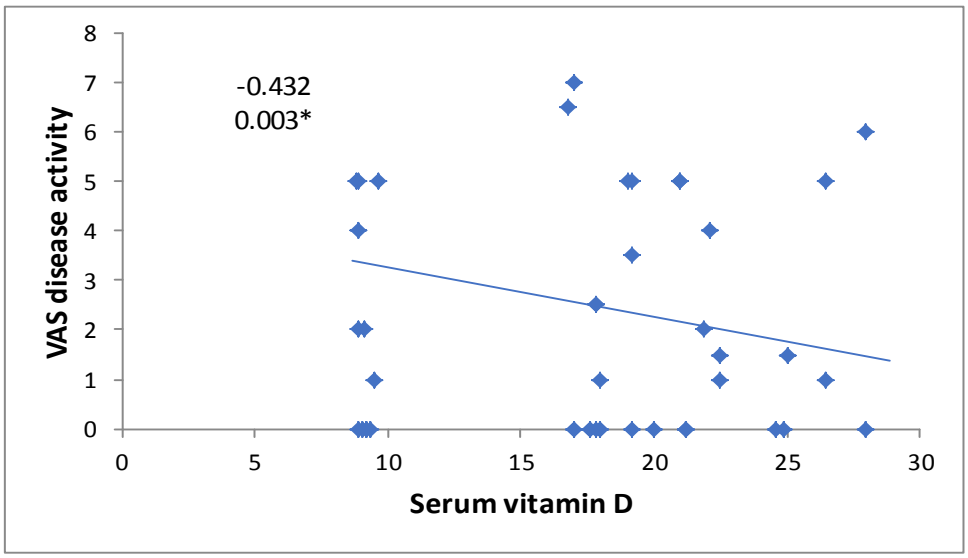

Figure (6): Correlation between vitamin D level and VAS disease activity.

DISCUSSION

JIA is one of the most familiar chronic inflammatory diseases affecting children under age of 16 years. The distinctive characters of the disease is asymmetric, peripheral polyarthritis. The etiology of JIA is still obscure; though a lot of environmental and genetic factors have been incriminated in the pathogenesis of the disease. So far many studies which had been performed suggested that vitamin D deficiency increases the risk of JIA development. Vitamin D evidently interact with immune system through its effects on regulation and differentiation of many cells like lymphocytes, macrophages, and natural killer cells, in addition to interference with the cytokines production.[22] The issue of universal vitamin D deficiency has hitherto been studied substantially both in the population at developmental age and in adult patients with rheumatological diseases, but studies focusing on children with JIA are scanty. Moreover no single measure had been standard as a precise indicator for childhood JIA disease activity. While C-reactive protein and erythrocyte sedimentation rate are indicators of inflammation, they alone do not perfectly mirror the overall disease activity.[2] Many studies evaluated the relationship between vitamin D and disease activity.

In this study, 25(OH) vitamin D levels were measured in 45 JIA children and compared with 30 healthy children of matched age and sex. The mean serum 25(OH) vitamin D levels in all JIA patients was found to be significantly lower than that of healthy controls ($P < 0.013$).

Similar findings were reported by Omelchenko L.I, who found that serum level of 25(OH)D (18±0.84 ng/ml) was significantly lower in all JIA patients compared to the children of control group (39.98±3.11 ng/ml), with the lowest serum concentrations in patients with high disease activity.[23] The same was reported by Cen et al. in their study,[24] where the mean serum Vitamin D level was

significantly lower in RA patients (35.99 ± 12.59 nmol/L) as compared to the normal participants (54.35 ± 8.20 nmol/L). Similarly, the study of Stark et al[25] reported that up to 50% of the patients with JIA had values below 30ng/ml . McNally et al[26] Showed that 82% of children with unexplained arthralgia had a low serum 25(OH)D level with 42% described as having insufficient level and 40% being deficient. The later two studies[25,26] reported a high prevalence of vitamin D deficiency in JIA; however, the results depend upon the definition of the "normal range" of Vitamin D. Szymanska-Kaluza et al[27] had detected statistically significant increase of $1,25(OH)_2D$ serum concentration in children with JIA compared to the control group.

On the contrary, Munekata et al[28] found high frequency of 25(OH)D insufficiency and deficiency in children with JIA; but there was no statistically significant difference between patients and controls. Lien et al[29] and Hillman et al[30] reported low serum level or vitamin D in both patients and controls. Possible interpretation for the high frequency of insufficiency and deficiency in both groups may be the increase use of sunblock's, dietary and urban lifestyles and the season when analyses had been undertaken.[31]

With respect to disease activity assessed according to the value of DAS28 score. There was a significant inverse relationship between serum vitamin D levels and JIA disease activity. There was statistically significant decrease in vitamin D serum level in patients with high disease activity compared to those with low activity; and this may be because the glucocorticoids intake is suspected to complicate the interpretation of the results as explained by Knight et al.[32]

Studies by Yassin *et al*[33], Azzeh and Kensara[34] observed the same results in Egyptian and Saudi patients with rheumatoid arthritis respectively and concluded that Vitamin D insufficiency is highly prevalent and linked to disease severity in patients with RA. A recent meta‑analysis also showed a significant inverse correlation between the Vitamin D levels and DAS28.[35] Leventis et al[36] reported in their study that Vitamin D supplementation in patient group may reduce RA disease activity.

The other way around Szymanska-Kaluza et al[27] demonstrated a higher mean concentrations of the active form of vitamin D in the serum of children with low activity of the disease compared to medium and high activity, but without statistical significance. Pelajo et al[37] found insignificant negative correlation between serum 25(OH)D levels and JIA disease activity, evaluated by JADAS-27. Carrasco et al[38] in their study detected no changes in the parameters of disease activity following Vit D supplementation in patients with JIA. Moreover, Higgins et al[39] reported that there was no significant correlation between vitamin D and DAS28.

The dissension in the previous studies may be explained by different statistical analytic methods used to detect the relation between vitamin D and disease activity. In addition to different scores used by some studies to assess the disease activity such as JADAS-27 in study of Pelajo et al[37] and DAS28 and VAS in study of Higgins et al.[39]

The present study showed significant difference in vitamin D level between JIA categories. Cases with systemic onset JIA had the lowest mean level compared to polyarticular and oligoarticular categories (p=0.016).

In the study of Bianchi et al[40] the polyarticular and systemic groups revealed significant decreased levels of 25(OH)D compared to the oligoarticular group. Similarly, the meta-analysis of Nisar et al[41] demonstrated that patients with systemic JIA were shown to have lower levels of both 25(OH) D and 1, 25(OH) D; however, there was no significant difference between the oligoarticular and polyarticular subgroups in this meta-analysis.

In opposition to ours, the analyzed results of studies by Falcini et al[42] and Reed et al[43] showed that Vit D levels were similar in all subtypes groups. Also, the difference in average vitamin D levels for those with polyarticular JIA and oligoarticular JIA was not significant in the study of Miettinen et al.[44] Szymanska-Kaluza et al[27] demonstrated higher mean concentrations of the active form of vitamin D and 25(OH)D in the serum of children with oligoarticular disease but without statistical significance.

Corticosteroids are particularly used in the treatment of SOJIA, especially cases with severe polyarthritis[45], which decrease level of 25(OH)D through disruption of vitamin D activation[46], this could explain the lower vitamin D level especially in this category of JIA patients.

Similar controversies are still present regarding serum alkaline phosphatase and PTH levels in JIA. We did not find any statistically significant difference between patients and controls regarding the serum levels of calcium, phosphorus, magnesium, alkaline phosphatase, and PTH. Only a positive correlation between DAS and both PTH and alkaline phosphatase levels was found. In contrast, Hillman et al.[30] found that, in JRA children not receiving steroids, serum levels of PTH were lower than in controls, while Bianchi et al.[40] showed increased values of PTH in JRA patients and proposed an impairment of hepatic 25-hydroxylation of vitamin D induced by steroid, as an explanation of this finding.

There are many explanations for these controversial results among different researcher work's concerned with this topic with the lack of statistical significance. It is suggested that the most important of these explanations are related to the differences in the season of measurement of vitamin D, the variable types of treatment lines which are not detected in most of the reviewed articles, and the JIA categories. These variables

can make it difficult to interpret the link between vitamin D and disease activity in relation to the inflammation status and the risk of relapse.[2]

We tested also the quality of life of JIA cases by using the scores: JAFS and VAS for well-being & for disease activity and both correlated negatively with the mean vitamin D serum level. Many studies reported inverse significant associations between vitamin D and related activity indices.[47]

CONCLUSION

Even though lower vitamin D levels in children with JIA, judgment is problematic, as still no definite definition of vitamin D deficiency exists in this category. Uniformity of vitamin D levels in the pediatric population, and specifically in JIA is urgently needed since vitamin D deficiency may be linked to disease severity, and could be correlated with duration of the disease Further studies with larger sample set of new-onset JIA patients, having more details about vitamin D intake, should be performed to confirm these results. The mostly affected JIA categories in vitamin D deficiency are the systemic and the polyarticular forms, therefore these disorders should be screened for vitamin D deficiency at regular intervals, and vitamin D supplementation in JIA are recommended.

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