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RESEARCH ARTICLE

THE ROLE OF VITAMIN D DEFICIENCY ON INFLAMMATORY MARKERS AND LEFT VENTRICULAR FUNCTIONS IN EGYPTIAN RACHITIC INFANTS

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ABSTRACT

Background: Circulating 25 hydroxyvitamin D (25 (OH)D), an accurate measure of vitamin D status, is markedly reduced in rachitic infants**.** Aside from the known relationship between vitamin D and bone, vitamin D has also been implicated in cardiovascular homeostasis, immune function and inflammation. Furthermore, a mass of evidence is accumulating that vitamin D deficiency could lead to cardiovascular complications and imbalance of cytokines profile. Our objective was to study the relationship between vitamin D status (as determined by serum 25(OH) D concentrations) and inflammatory markers and left ventricular function in rachitic infants. Also, to evaluate the effect of vitamin D supplementation on the above parameters.

Subjects and methods: This study included two groups; vitamin D deficiency rickets (VDDR) group (25 infants) and an age matched control group (15 infants). After subsiding of the acute illness, the rachitic infants received vitamin D supplementation for 6 months. Blood samples were collected in the morning before the start of treatment and analyzed for serum 25(OH)D, intact parathyroid hormone (iPTH), Alkaline phosphtase (ALP), calcium (Ca), Phosphorus (Ph) and inflammatory markers (interleukin-6 (IL-6), and C-reactive protein (CRP)). Electrocardiogram (ECG) and echocardiography measuring left ventricular functions were done. The biochemical variables, ECG and echocardiography were assessed at baseline and after 6 months of vitamin D supplementation.

Results: VDDR group had significant lower 25(OH)D, Ca, Ph and significant higher iPTH, ALP, IL-6 and CRP compared to the age matched control group at baseline. Echocardiographic finding revealed significant increase in LVEDD and LVESD and significant decrease in EF% and FS% in VDDR group compared to the age matched control group at the study entry. Also, ECG finding showed abnormality in some patients at baseline. The biochemical, echocardiogrphic and ECG variables improved significantly after 6 months of vitamin D supplementation and reached to those levels found in the age matched control group. Finally, we found negative correlations between 25(OH)D level and IL-6, CRP, LVEDD and LVESD. Also, positive correlations were found between 25 (OH)D and EF% and FS%. These correlations were observed at baseline and after 6 months of vitamin D treatment.

Conclusion: VDDR is associated with increased inflammatory markers and impairment of left ventricular functions in rachitic infants. Vitamin D supplementation ameliorated these effects. Also, results gleaned from this investigation support the possible contributing role of the elevated inflammatory markers in the pathophysiology of left ventricular impairment in vitamin D deficiency rachitic infants. More studies are needed to fully characterize the relationship between Vitamin D induced inflammation and cardiac function in rachitic infants.

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INTRODUCTION

Nutritional rickets is a disease resulting from impaired bone mineralization due to insufficient calcium or phosphorus in growing children. It ranks as one of the five commonest diseases in children from developing countries and is still quite common in the Middle East (Thacher *et al*., 2006). It is thought to be secondary to vitamin D deficiency (Baroncelli *et al*., 2008). Several studies have shown that vitamin D may play a role in many biochemical mechanisms in addition to bone and calcium metabolism. Recently, vitamin D has sparked widespread interest because of its involvement in the homeostasis of the cardiovascular system (Muscogiuri *et al*., 2012; Jinghui Dong *et al*., 2014). There is growing evidence

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that vitamin D either directly or indirectly affects cardiac structure and function (Pilz *et al*., 2010; Mozos and Marginean 2015). The vitamin D receptor knockout mouse model demonstrates marked cardiomyocyte hypertrophy and increased left ventricular weight (Chen *et al*., 2011), and $1,25(OH)_2D_3$ attenuates cardiomyocyte proliferation (Nibbelink *et al*., 2007) and hypertrophy (Wu *et al*., 1996) in vitro. In human, Vitamin D deficiency has been shown to be associated with an increased incidence of left ventricular hypertrophy and congestive heart failure (Kunadian *et al*., 2014). Although it has been reported that asymptomatic left ventricular dysfunction may develop in infants with vitamin D deficiency rickets (VDDR) and it improves with treatment, dilated cardiomyopathy and congestive heart failure are rare (Verma *et al*., 2011). There is increasing evidences that low vitamin D status may lead to immune dysregulation. Studies have shown defective macrophage function, such as impaired chemotaxis, phagocytosis, and increased production of proinflammatory cytokines in vitamin D deficiency (Arnson *et al*., 2013). Vitamin D supplementation improved cytokines profiles in animals (Canning *et al*., 2001; Zhou *et al*., 2008), patients with congestive heart failure (Patel and Rizvi, 2011; Debreceni and Debreceni, 2014) and human coronary arterial endothelial cells (17,18,19). To date there is little evidence on the associations of 25(OH)D with indicators of inflammation and cardiac functions in rachitic infants. So, the aim of this work was to evaluate the effect of vitamin D deficiency on the inflammatory markers; interlukin-6 (IL-6) and C-reactive protein (CRP) and the left ventricular function in the rachitic infants. Also, we examined the effect of vitamin D supplementation on the above mentioned parameters. Moreover, we searched for potential correlations between 25(OH)D and IL-6, CRP and selected echocardiographic parameters.

MATERIALS AND METHODS

Participants

This study included 40 infants with age range from 6 months to 2 years. 25 (14 boy and 11 girls) infants with vitamin D deficiency rickets (VDDR). 15 (9 boys and 6 girls) apparently healthy, age matched infants were studied as a control. Both patients and controls were recruited from Paediatric Outpatients Clinics and Paediatric Emergency department in Aswan University Hospital, Egypt. The diagnosis of VDDR was based on a combination of clinical, radiographic and biochemical features of VDDR (Hatun *et al*., 2005). Exclusion criteria were previous history of heart disease or any other condition that affect cardiac functions, history of prematurity or intrauterine growth retardation, renal, liver, intestinal or central nervous system disease, family history of hereditary forms of rickets, treatment with vitamin D, malnutrition and anemia. The work was approved by the Aswan University Ethics Scientific Committee and an informed consent from the parents of infants had been performed. At the study entry, blood samples were taken from all patients (VDDR group) and then received intramuscular injection of vitamin D (cholecalciferol) (600 000 IU) once and oral calcium lactate for 2 weeks followed by oral maintenance dose of vitamin D 400 unit/day for 6 months.

A) Biochemical analysis

Blood samples were drawn in the morning between 8 AM and 11 AM at baseline and at the end of the 6 months of treatment. After centrifugation at room temperature for 20 minutes, aliquots of the serum samples were frozen consecutively and stored at -20 °C until analyzed. The following biochemical parameters were measured using ELISA kits: IL-6 (AviBion Human IL-6 ELISA kit, Orgenium Laboratories, Finland), C-reactive protein (highly sensitive CRP ELISA Kit Monobind Inc., USA). 25-hydroxyvitamin D (25OHD) was measured using enzyme immunoassay (Immunodiagnostic Systems Inc., Fountain Hills, AZ) and intact parathyroid hormone (i-PTH) was measured using immunoassay (Immulite 1000, Diagnostic Products Corporation). alkaline phosphatase was determined using Abbot Aeroset Autoanalyzer by spectrphotometric method. Ca and Ph levels were measured using routine laboratory tests.

B) Electrocardiographic Measurements

Resting12-lead electrocardiograms (ECG) studies were performed for all rachitic cases and interpreted in accordance with the patient's age and sex, and the QT segment was corrected for heart rate (QTc) (Park, 2008).

C) Echocardiography

After improvement of acute illness of studied cases. For all patients and controls, left ventricle functions were evaluated by echocardiography using Vivid 3, Aloka machines with transducers of 3.5,7 MHz. We used different echocardiography Modes: 1) two dimensional (2D) to verify cardiac chambers structures and details of anatomy. 2) M mode study to estimate the other echocardiographic variables according to the criteria of the American Society of Echocardiography (Brunvand *et al*., 1995).

Statistical analysis

Data are expressed as mean ± standard deviation (SD) for all parameters. The data were analysed by using Graph Pad Prism data analysis program (GraphPad Software, Inc., San Diego, CA, USA). For the comparison of statistical significance between cases and control, Student Newman-Keuls t-test for unpaired and paired data was used. Linear correlations were performed by Spearman's or Pearson's test. A value of $P \leq 0.05$ was considered statistically significant.

RESULTS

The biochemical and echocardiographic variables of the two groups at the baseline are summarized in Table 1. patients with VDDR had significantly lower level of serum Ca, Ph and 25(OH) vitamin D (for all $P<0.001$) and significantly higher level of alkaline phosphatase, parathyroid hormone, IL-6 and CRP (for all $P<0.001$) in comparison with age matched control group. The echocardiographic parameters of VDDR group; LVEDD and LVESD, were significantly higher (for both $P<0.001$) while EF% and FS% were significantly lower (for both $P<0.001$) when compared to the control group. No

significant difference in IVSWT, LVPWT, I/L, LVM, LVMI and E/A between VDDR group and the control group. After 6 months of treatment (shown in table 2), the serum Ca, Ph and 25(OH) vitamin D of the VDDR group were significantly higher (for all P<0.001) compared to the levels found at the baseline. Alkaline phosphatase, parathyroid hormone, IL-6 and CRP levels of VDDR participants after 6 months of treatment were significantly lower (for all P<0.001) compared to levels showed at baseline. All of these parameters return to normal levels and were not significantly different when compared to an age matched control group. The echocardiographic variables; LVEDD and LVESD, were significantly lower (for both P<0.001) while EF% and FS% were significantly higher (for both P<0.001) when compared to the baseline levels. These variables were not significantly different compared to the age matched control group. ECG of VDDR group showed T wave abnormalities in 3 cases and prolonged QT interval in 5 cases at the baseline. These changes disappeared after 6 months of vitamin D supplementation (data not shown).

Correlation analysis

Figures 1 (A&B), 2 (A&B) showed correlation coefficient between 25 (OH) vitamin D level and IL-6, CRP, echocardiographic variables: LVEDD, LVESD, EF% and FS% among VDDR group at baseline and after 6 months of treatment. At baseline, serum 25 (OH) vitamin D level had significant -ve correlation with IL-6 (r= - 0.68 and P<0.001), CRP (r= -0.59 and P<0.01), LVEDD (r= - 0.66 and P<0.001), LVESD (r= - 0.79 and P<0.001) and significant +ve correlation with EF% (r= 0.71 and P<0.001) and FS% (r= 0.69 and P<0.001). After 6 months of treatment, serum 25 (OH) vitamin D level had significant -ve correlation with IL-6 (r=- 0.94 and P<0.001), CRP (r= -0.53 and P<0.01), LVEDD (r= - 0.69 and P<0.001), LVESD (r= - 0.77 and P<0.001) and +ve correlation with EF% (r= 0.87 and P<0.001) and FS% (r= 0.56 and P<0.01). Quantitative variables are expressed as mean±SD, student t test were used to compare between the two groups.

Table 1. Biochemical and echocardiographic variables of the study groups at baseline

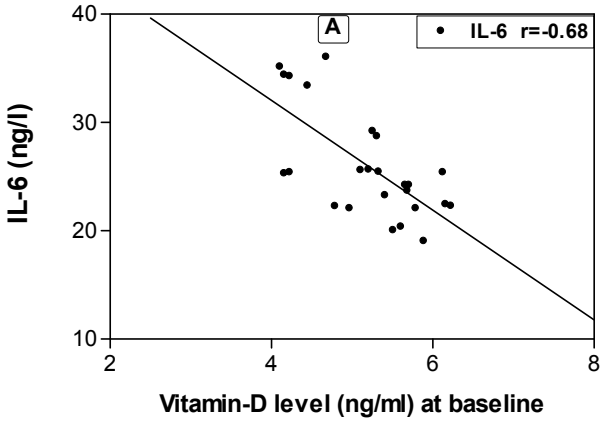
Variables	VDDR group N=25	Control group N=15	P value
Calcium (mg/dl)	7.1 ±0.6	9.1 ±0.6	P<0.001
Phosphorus (mg/dl)	1.8 ±0.6	5.5 ±0.8	P<0.001
ALP (IU)	490 ±50.2	142 ±31.6	P<0.001
iPTH (pg/ml)	212.8±25.3	44±6.6	P<0.001
25(OH) vitamin D (ng/ml)	5.18±0.68	24.5±1.4	P<0.001
CRP (mg/l)	14.01±1.73	5.35±1.42	P<0.001
IL-6 (ng/ml)	26.07±5.01	6.08±1.3	P<0.001
LVEDD (mm)	31.92±5.01	22.14±2.56	P<0.001
LVESD (mm)	23.57±1.15	15.35±1.82	P<0.001
EF (%)	57.41±3.43	65.36±7.86	P<0.001
FS (%)	23.96±0.99	36.25±2.4	P<0.001
IVSWT (mm)	3.89±0.42	4.16±0.54	NS
LVPWT (mm)	3.75±0.5	3.82±0.5	NS
I/L	1.06±0.06	1.04±0.04	NS
LVM (g)	30.77±4.2	31.22±2.85	NS
LVMI (g/m^2)	67.4±11.5	65.83±14.5	NS
E/A	1.33±0.05	1.35±0.08	NS

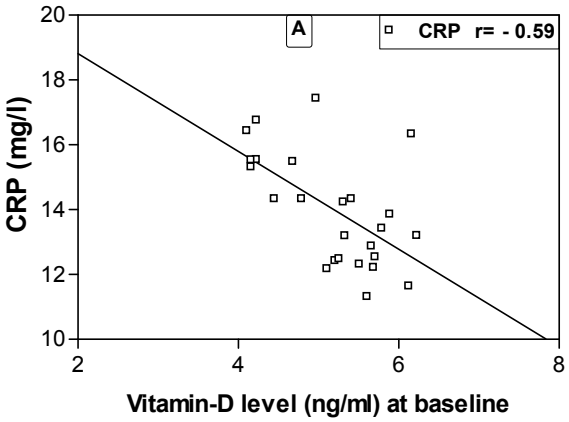
ALP: alkaline phosphtase; iPTH: intact parthyroid hormone; CRP: C-reactive protein; IL-6: interlukin-6; LVEDD: Left ventricular end diasolic diameter; LVESD: left ventricular end systolic diameter; FS: fractional shortening; EF: ejection fraction; IVSWT: interventricular septal wall thickness; LVPWT: left ventricular posterior wall thickness; I/L: interventricular posterior wall thickness / left ventricular posterior wall thickness; LVM: left ventricular mass; LVMI: left ventricular mass index; E/A ratio: E wave /A wave ratio.

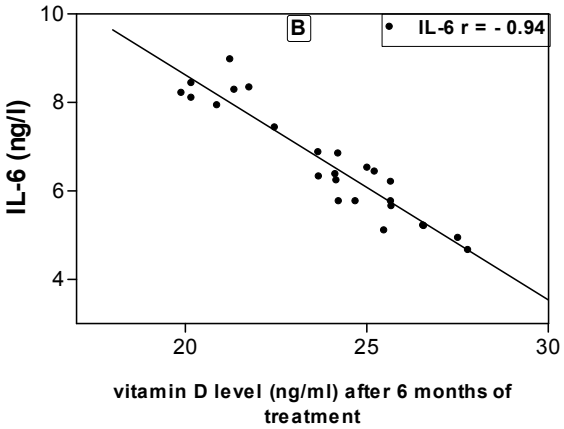
Table 2. Biochemical and echocardiographic variables of the study groups at baseline, after 6 months of treatment

Variable	VDDR at base line	VDDR after 6 month of treatment	Control	P value	
				At base line vs after 6 months of treatment	After 6 months vs control
Calcium (mg/dl)	7.1 ±0.6	9.2 ±0.6	9.4 ±0.8	P<0.001	NS
Phosphorus (mg/dl)	1.8 ±0.4	5.00±0.6	5.2 ±0.8	P<0.001	NS
ALP (IU)	490 ±50.2	172±58.6	155 ±42.6	P<0.001	NS
iPTH (pg/ml)	212.8±25.3	47.5±7.2	44.6±6.6	P<0.001	NS
25(OH) vitamin D (ng/ml)	5.18±0.68	23.9±2.34	24.12±1.95	P<0.001	NS
CRP (mg/l)	14.01±1.73	6.08±1.08	5.65±1.12	P<0.001	NS
IL-6 (ng/l)	26.07±5.01	6.64±1.27	6.08±1.3	P<0.001	NS
LVEDD (mm)	31.92±1.04	20.37±2.55	21.1±2.1	P<0.001	NS
LVESD (mm)	23.57±1.15	14.26±1.21	13.98±1.5	P<0.001	NS
EF (%)	57.41±3.43	64.06±6.1	66.5±8.6	P<0.001	NS
FS (%)	23.96±0.99	34.08±3.73	35.9±3.9	P<0.001	NS
IVSWT (mm)	3.89±0.42	3.96±0.45	4.1±0.6	NS	NS
LVPWT(mm)	3.75±0.5	3.8±0.82	3.9±0.6	NS	NS
I/L	1.06±0.06	1.07±0.07	1.05±0.04	NS	NS
LVM (g)	30.77±4.2	31.65±3.4	32±3.9	NS	NS
LVMI (g/m^2)	67.4±11.5	65.43±11.7	64.76±12.4	NS	NS
E/A	1.33±0.05	1.35±0.09	1.34±0.07	NS	NS

Quantitative variables are expressed as mean±SD, student t test were used to compare between the two groups. ALP: alkaline phosphtase; iPTH: intact parthyroid hormone; CRP: C-reactive protein; IL-6: interlukin-6; LVEDD: Left ventricular end diasolic diameter; LVESD: left ventricular end systolic diameter; FS: fractional shortening; EF: ejection fraction; IVSWT: interventricular septal wall thickness; LVPWT: left ventricular posterior wall thickness; I/L: interventricular posterior wall thickness / left ventricular posterior wall thickness; LVM: left ventricular mass; LVMI: left ventricular mass index; E/A ratio: E wave /A wave ratio.







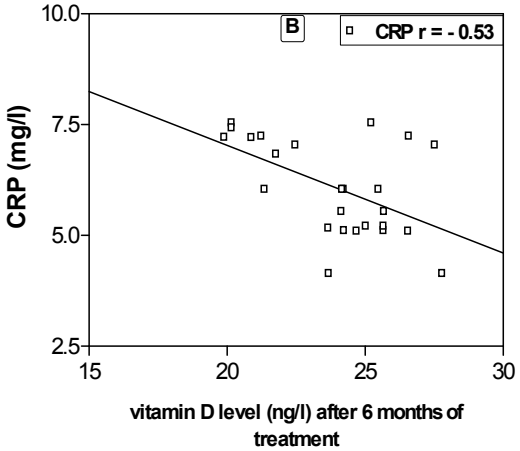
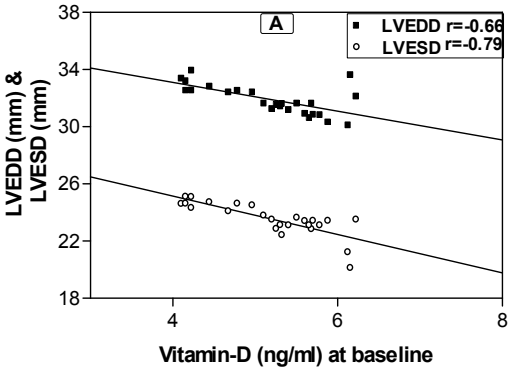
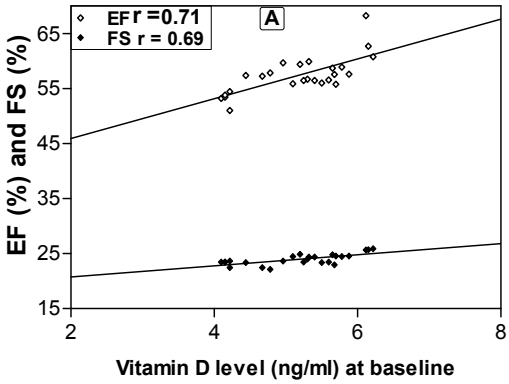
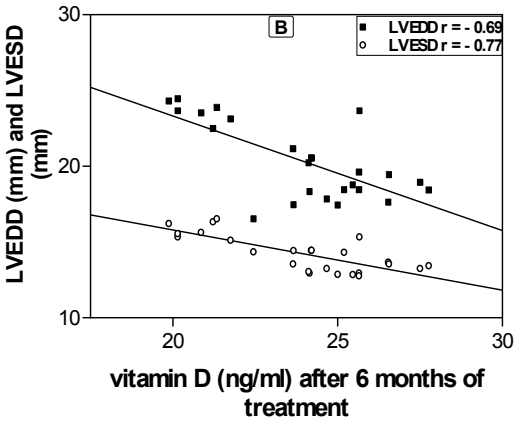
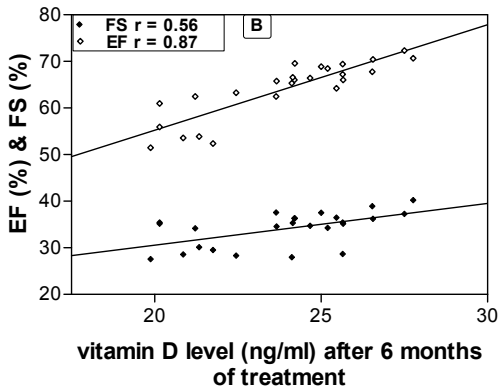


Figure 1. A and B correlation coefficients between Vitamin D and IL-6 and CRP in VDDR group at baseline (A) and after 6 months of treatment (B)









The EF r =0.56
FS r=0.87

Figure 2. A and B correlation coefficients between Vitamin D and each of echocardiographic parameters; LVEDD: Left ventricular end diasolic diameter; LVESD: left ventricular end systolic diameter; FS: fractional shortening; EF: ejection fraction in VDDR group at baseline (A) and after 6 months of treatment (B)

DISCUSSION

Vitamin D has received worldwide attention not only for its importance for bone health in children and adults but also for reducing risk for many chronic diseases including autoimmune diseases, type 2 diabetes, heart disease, many cancers and infectious diseases (Jinghui Dong *et al*., 2014; Holick, 2012). Vitamin D has net effect of increasing serum levels of calcium and phosphorus levels and achieves this by increasing intestinal calcium and phosphorus absorption. Vitamin D deficiency results in reduced serum calcium, which triggers secretion of parathyroid hormone to release calcium and phosphorus from bone in an attempt to maintain normal serum calcium levels (Fauci *et al*., 1998). Regarding the cardiovascular system, investigators have found an association between vitamin D deficiency and cardiovascular diseases and risk factors (Gunta *et al*., 2013; Wranicz and Szostak-Węgierek, 2014; Chowdhury *et al*., 2014; Annuzzi *et al*., 2012). Vitamin D reduces the expression of several genes which are upregulated in myocardial hypertrophy, e.g. by suppressing the cardiac rennin-angiotensin system and natriuretic peptides. Vitamin D has been shown to exert antihypertrophic effects on cardiomyocytes by increasing thrombomodulin and decreasing tissue factor (Oz *et al*., 2013; Pandit *et al*., 2014). Also, vitamin D exerts various effects on the growth and differentiation of cardiomyocytes, which are largely suggested to improve myocardial structure and function. In addition, it has been shown that cardiac myocytes and fibroblasts express the enzymes 1α-hydroxylase (Adams and Hewison, 2012). Furthermore, the expression of myosin, a major contractile protein of the myocardium, is also regulated by vitamin D which may explain the associations of vitamin D status and myocardial contractility (Wacker and Holiack, 2013). In the present study, serum Ca level was low in VDDR at baseline compared to the control group and reach to the normal level after 6 months of treatment with vitamin D. Within the heart, calcium ions are essential for the initiation of excitation-contraction coupling via an influx through L-type calcium channels. Once it is released from the sarcoplasmic reticulum by ryanodine receptors, calcium determines contractility by mediating the tension developed between actin and myosin filaments via the troponin-tropomyosin complex. Decreased amounts of available calcium lead to diminished responses in both of these areas and decreased cardiac function (Opie, 2001). In the present work, PTH levels were high in VDDR group at baseline and decreased after 6 months of vitamin D treatment. As 25 (OH) vitamin D falls, intestinal absorption of calcium falls leading to decreased serum calcium. This causes a rise in the serum PTH, which stimulate conversion of 25 (OH) D to 1,25 (OH)2 D and thereby maintains absorption of calcium (Durazo-Arvizu *et al*., 2010). Thus optimal level of 25 (OH) D is defined as level which causes maximal suppression of PTH and maximum Calcium absorption (Sahay and Sahay, 2012; Savica *et al*., 2013). Elevated PTH was level reported to be a cardiovascular risk factor independent of calcium and phosphorus levels (Lishmanov *et al*., 2012; van Ballegooijen *et al*., 2013). PTH is pro-atherosclerotic, stimulates systemic and vascular inflammation, augmenting atherogenesis (Kienreich *et al*., 2013; Carbone *et al*., 2014). Also, high PTH levels activates the renin-angiotensin system, causing increased blood pressure and left ventricular hypertrophy (with subsequent apoptosis and fibrosis). It is debated whether the beneficial effects of vitamin D on the cardiovascular system are direct or related to the physiological vitamin D-related lowering of PTH levels (Abu *et al*., 2013).

Results of the present study showed a decrease in phosphorus level at baseline of VDDR group which improved after treatment. Liu *et al*. (2009) reported that hypophosphatemia caused left ventricular hypertrophy with upregulation of catecholamine and renin-angiotensin system components. Also, a previous study illustrated that the hypophosphatemia that resulted from vitamin D deficiency resulted in muscle weakness (Schubert and Deluca, 2010). They suggested that the muscle weakness could result from central importance of phosphorus in muscle function involving large amounts of ATP and the high level of phosphorylation and dephosphorylation of proteins during contraction and relaxation. In our study, alkaline phosphatase (ALP) level was high at the study entry of the VDDR group which was normalized after 6 months of vitamin D supplementation. ALP is an excellent marker of rickets activity because it participate in the mineralization of bone and growth plate cartilage. Serum ALP is elevated in hypocalcemic rickets (Whyte, 2010). Sahay and Sahay (2013) suggested that ALP may be used for the screen of rickets. In our study, serum IL-6 and CRP levels were elevated at the baseline and reached the normal levels after 6 months of vitamin D treatment. Alterations in the inflammatory markers with vitamin D deficiency were observed by many investigators (Jamali *et al*., 2012; Ferder *et al*., 2013; van de Luijtgaarden *et al*., 2012; Thota *et al*., 2012; Mangin *et al*., 2014). Thota *et al*. (2012) showed that vitamin D caused down regulation of IL-6 and up regulation of anti-inflammatory cytokines. Also, Beilfuss *et al*. (2012) found that 1 year of vitamin D supplementation reduces the level of IL-6 in vitamin D deficient subjects. In addition, a study done on infants with congestive heart failure who have baseline 25-hydroxyvitamin D below the lower end of the reference range, 12 weeks of vitamin D supplementation resulted in improvement of LVEDD, LVESD, EF%, FS% and decreased IL-6 level (Shedeed, 2012; Witham *et al*., 2014). He suggested that vitamin D is a potent anti-inflammatory agent that improved cytokine profile balance. Moreover, experimental evidences has been identified that vitamin D deficiency induced hypertrophy in cardiomyocytes with decreased expression of vitamin D receptor and suppressor of cytokine signaling (SOCS3) in cardiomyocyte which was also associated with increased inflammatory markers in epicardial adipose tissue (Gupta *et al*., 2012). Liss and Fisherman (Liss and Frishman, 2012) proposed that increment of proinflammatory cytokines tumor necrosis–α (TNFα) and IL-6 are one of the pathophysiological mechanisms involved in heart disease with vitamin D deficiency.

In the present study, EF% and FS% were lower while LVEDD and LVESD were higher in VDDR group at the baseline and normalized after 6 months of treatment. EF% and FS% are the most commonly used parameters in the clinical evaluation of systolic functions of the left ventricle (Ocall *et al*., 2001). This indicated the presence of systolic dysfunction and poor left ventricular contraction at baseline that reach normal values after treatment. Also, increased LVEDD and LVESD signified

the presence of dilated left ventricle among the studied subjects. The combination of dilated left ventricle and poor contractility of left ventricle implying dilated cardiomyopathy among VDDR subjects (Pilz *et al*., 2013). Some investigator reported that VDDR caused asymptomatic left ventricular dysfunction that improves with treatment. They concluded that VDDR must the considered as an important curable cause for dilated cardiomyopathy among children especially in regions where nutritional rickets is still common (Verma *et al*., 2011; Ford *et al*., 2014). Finally, our results demonstrated significant –ve correlations in VDDR group between Vitamin D and each of IL-6, CRP levels, LVESD and LVEDD at baseline and after 6 months of treatment. On the other hand, significant +ve correlations were observed between vitamin D and FS% and EF%. These results are in line with Eleftheriadis *et al*. (2012) who found inverse correlation between Vitamin D and IL-6 and CRP and Fall *et al*. (2012) who observed higher circulating vitamin D concentrations to be associated with better left ventricular systolic function and smaller LVESD. This means that the increment of vitamin D concentration in VDDR is associated with improvement of cytokines profile and left ventricular function. In conclusion, Vitamin D deficiency in rachitic infants is associated with increment of inflammatory markers and left ventricular impairment. Vitamin D supplementation in rickets reduce the cardiovascular complication and improve the associated systemic inflammation. Also, our results support the concept of a possible contributing role of the elevated inflammatory markers in the pathophysiology of impaired left ventricular function in vitamin D deficient rachitic infants.

Conflict of Interest

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/ or affiliations relevant to the subject matter or materials included.

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