Original Article

VITAMIN D STATUS AND SERUM LEVEL OF SOME ELEMENTS IN CHILDREN WITH SICKLE CELL DISEASE IN JEDDAH, SAUDI ARABIA

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ABSTRACT:

Objective: To study the relationship of Vitamin D deficiency and some minerals metabolism in the children with sickle cell disease (SCD) in the city of Jeddah, western region of Saudi Arabia.

Design: Measuring the concentration of serum 25(OH) Vitamin D, calcium, phosphorus and magnesium in children with SCD aged between newborn to 12 years old.

Methods: A total of 51 children with sickle cell disease (both gender) included 28 males (54.9%) and 23 females (45.1%) aged between newborn and 12 years old and 70 healthy matching controls were admitted or visited sickle cell section in the Maternity and Children Hospital in the city of Jeddah. Fasting blood samples were collected and the serum was separated and stored at –30ºC until the time of analysis. Serum 25 (OH) Vitamin D was determined using a commercially available kit (VDBP,Gc globulin), calcium, phosphorus and magnesium were measured using a clinical autoanalyser.

Results: The patients were divided into two groups according to the ages. Group-A included 21 patients (both gender) aged between newborn and 6 years, group-B included 30 patients (both gender) aged between 7-12 years. The results obtained showed that the serum concentrations of 25(OH) Vitamin D in both patients groups were significantly lower than the healthy matching controls ($P<0.01$ and $P<0.001$), respectively. The serum concentrations of calcium, phosphorus and magnesium in group-A patients had no significant differences, whereas in group-B, the concentration of Ca^{+2} was significantly lower in patients than the controls ($P<0.05$), no significant differences in P in both groups ($P>0.05$) and significantly higher in the serum magnesium of group-B ($P<0.05$).

Conclusion: A significant relation between Vitamin D deficiency and children with sickle cell disease which is normal due to confined patients indoor. The serum calcium concentration had no affect in the early stage of ages but a significant lower appeared with increasing of ages. The serum magnesium concentration was higher in group-B which can be explained to the important role of Mg^{+2} in the nature of erythrocyte membrane in sickle cell patients.

KEY WORDS: Sickle cell disease, 25-hydroxyvitamin D, Vitamin D, Binding protein assay, Parathyroid hormone.

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INTRODUCTION

Sickle cell disease (SCD), a prototypical molecular disorder is an inherited disease that affects red blood cells.[1-3] People with sickle cell disease have red blood cells that become hard and pointed instead of soft and round. The disease is a global health problem. In Saudi Arabia, sickle cell and glucose 6-phosphate dehydrogenase deficiency (G6PD) are among the major health problems and constitute the most genetic disorders among people originally belonging to the eastern province of the kingdom.[4-8]

The prevalence in infants with homozygous sickle cell in Qatif and AL-hasa cities (Eastern region of the kingdom) was detected in 2.35%

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and 1.08% respectively, and the frequency of sickle cell gene was 0.1545% and 0.1109%.[9] The combination of sickle cell anemia and Vitamin D deficiency is seldom reported in literatures, especially in Arab countries. Vitamin D synthesized in the skin and absorbed in the intestine, undergoes metabolic transformations before exerting its effect on target tissues.[10,11] There are two different forms of Vitamin D, namely D_3 and D_2, which are structurally very similar. The latter one is synthetic product, which is predominantly absorbed via fortified food, physiological Vitamin D_3 levels result from dietary uptake but also from biosynthesis in the skin from 7-dehydrocholesterol and UV-light due to sun exposure.[12-16] The Vitamin is then hydroxylated in the liver to 25-hydroxyvitamin D (25-OH Vitamin D), which is the major circulating metabolite of Vitamin D. Although the biological active form of Vitamin D is 1,25 $(OH)_2$ Vitamin D, synthesised in the kidney, it is widely accepted that measurement of circulating 25(OH) Vitamin D provides better information with respect to the patients' Vitamin D status and is used for diagnosis of hypovitaminosis.[17] At all ages from neonates to older people, lower serum levels of 25(OH) Vitamin D are associated with higher levels of PTH.[18,19] A deficiency of Vitamin D has been shown to reduce calcium absorption, increase PTH excretion thereby stimulating osteoclastic activity and thus increasing bone loss.[20] Mohammed et al.[21] reported that 5-25 years old patients with SCD in the Southern region of Saudi Arabia have low serum calcium and 25(OH) Vitamin D and high serum PTH compared with age and sex matched controls. The finding of Mohammed *et al*.[21] suggests that patients in the Southern region of kingdom with SCD and low Vitamin D status contribute to hypocalcaemia, a tendency for secondary hyper-parathyroidism and possibly skeletal abnormalities. The aim of this study was to investigate the effect of sickle cell disease (SCD) on Vitamin D status and the serum concentration of some elements in children with SCD in the city of Jeddah (Western region) of the Saudi Arabia kingdom.

PATIENTS AND METHODS

Fifty-one SCD patients of both sexes (28 males and 23 females) aged between newborn and 12 years were investigated. Seventy healthy matching controls (32 males and 38 females) served as control group. The patients and controls were divided into two age groups; group-A, from newborn to 6 years old were included 21 patients and 29 healthy matching controls. Group-B, from 7-12 years old were included 30 patients and 41 healthy controls. All patients were in the steady state (crises free for previous four months at the time of study). Neither the patients nor the controls were taking any Vitamin D supplements. Diet and exposure to sunlight were the main sources of Vitamin D.

Fasting blood samples were collected in plain tubes, and the serum was separated and stored at -30ºC until the time of analysis. Serum calcium, phosphate and magnesium were measured using a clinical technician auto-analyser (Tarrytown, USA). Serum sample 25(OH) Vitamin D was determined using a commercially available kit (VDBP, Gc globulin). It based on the competition of 25(OH) Vitamin D present in the sample with biotinylated 25(OH) Vitamin D (tracer) for the binding pocket of Vitamin D binding protein (VDBP, Dc globulin) and absorption was measured with ELISA reader at 450 nm. The results are expressed in ng / ml.[22]

Statistical anaylsis:

Data were analyzed using SPSS for WINDOWS (version 10.0; SPSS Inc. Chicago. USA). Values were expressed as mean ± standard deviation (SD). Differences were considered statistically significant at $P<0.05$.

RESULTS

The data analysis shows no significant difference between sexes. Therefore, the results are presented regardless of sex. Group-A which included 21 patients aged between newborn

to 6 years old in Table-I, shows that SCP have significant lower mean values in the serum concentration of 25(OH) Vitamin D (P<0.01) compared with healthy matching controls. The serum concentration of calcium, phosphorus and magnesium has no significant difference between patients and healthy matching controls (P>0.05).

The data in Table-II shows the results of group-B that included 30 patients aged between 7 to 12 years old. The serum concentration of 25(OH) Vitamin D is significantly lower (P< 0.001) than the matching controls. The serum concentration of calcium is significantly lower (P<0.05) than the controls, while serum phosphorus is slightly lower, but not significantly (P>0.05), the serum magnesium concentration is significantly higher in patients than the matching controls (P<0.05).

A comparison of 25(OH) Vitamin D level in the two groups, showed that the level of Vitamin D is higher in group-B than in group-A. This finding is normal observation on the basis that Vitamin D concentration increased with the increase of age which may be attributed to the daily dietary intake of Vitamin D and more exposure to the sunlight of this group than group-A.

Table-I: Serum concentration of Vitamin D, calcium, phosphorus and magnesium in group-A sickle cell patients

Subjects	*25(OH) Vitamin D (ng/ ml)*	*Calcium (mmol/l)*	*Phosphorus (mmol/l)*	*Magnesium (mmol/l)*
Patients (n = 21)	10.7 ± 0.09	2.55 ± 0.24	1.66 ± 0.05	0.76 ± 0.08
Controls (n = 29)	15.4 ± 0.55	2.6 ± 0.05	1.70 ± 0.07	0.78 ± 0.045
P value	(P < 0.01)	(P > 0.05)	(P > 0.05)	(P > 0.05)

± SD = Standard Deviation
The data is the mean values of triplicate.
Differences were considered statistically significant at P < 0.05.

Table-II: Serum concentration of Vitamin D, calcium, phosphorus and magnesium in group-B sickle cell patients

Subjects	*25(OH) Vitamin D (ng/ ml)*	*Calcium (mmol/l)*	*Phosphorus (mmol/l)*	*Magnesium (mmol/l)*
Patients (n = 30)	14.26 ± 0.28	2.3 ± 0.03	1.67 ± 0.04	0.90 ± 0.02
Controls (n = 41)	23.06 ± 0.25	2.6 ± 0.15	1.73 ± 0.12	0.79 ± 0.03
P value	(P < 0.001)	(P < 0.05)	(P > 0.05)	(P < 0.05)

± SD = Standard deviation
The data is the mean values of triplicate.
Differences were considered statistically significant at P < 0.05.

DISCUSSION

Sickle cell disease (SCD) is a genetic disorder caused by an alteration in the molecular structure of hemoglobin. The disease is highly prevalence in the eastern and southwestern of Saudi Arabia.[9,21,23,24] The present study was focused on the determination of Vitamin D level and some related mineral metabolism among infants and children with sickle cell disease in Jeddah city in the western region of the kingdom. The result presented shows that the serum concentration of 25(OH) Vitamin D is significantly lower in both groups A & B ($P<0.01$ and <0.001), respectively, than the matching healthy controls. It is clear from results that there are increasing values with age in controls and in patients that may be attributed to the low limit of daily dietary intake of Vitamin D and more exposure to the sunlight. The low levels of 25(OH) Vitamin D seen in our patients might be due to the various well-known malfunctions and structural changes of the liver in SCD. These changes might affect the rate of the hydroxylation step of Vitamin D, which may lead to decreased concentration of 25(OH) Vitamin D in the blood.[25,26] A previous studies on the status of Vitamin D in normal subjects from the middle region of Saudi Arabia (Riyadh area) had indicated that Saudis especially the elderly, had low levels of 25(OH) Vitamin D with no differences between males and females.[27,29] They concluded that the low level of Vitamin D were attributed to the low dietary intake of Vitamin D and also due to avoidance of sunlight. In our study it was not clear for us that the dietary intake of Vitamin D by both SCP and controls were adequate that there was no assessment of food frequency questionnaire of dietary recall interviews. The patients and controls in this study were mainly from Jeddah area (western region) of Saudi Arabia which where the chances for sunlight exposure is much greater than the Riyadh area. Therefore, since SCP is often ill and confined indoors, it is possible that they are less exposed to sunlight than their normal counterparts. The results in this study showed no significant differences regarding the level of calcium in either the patients or controls in group-A, whereas a significant lower level was seen in group-B. It was clear that the values of parameters in SCP were closer to the lower limit for both Vitamin D and calcium of the normal reference ranges (25(OH) Vitamin D; 14-44 ng/ml and calcium, 2.2-2.6 mmol/l).[30] A hypocalcaemia in SCP was reported by Nduke & Ekeke.[31] Others reported that the calcium content of sickle cells is about 10 fold higher than in normal erythrocytes, and that this is responsible for the destruction of sickle cells.[32-33] The serum phosphorus level was determined in this study and there were no significant differences neither in the patients and in the controls in both groups. Smith et al.[34] study showed that increased serum phosphate levels in some sickle cell patients. They concluded that increased levels of serum phosphate are related to alter renal handling of phosphate, which is associated with increased clearance of sodium, and of interest was the increased frequency of painful crises in patients with high levels of serum phosphate as compared to those with normal phosphate levels.

Serum magnesium concentration in group-A patients were slightly lower than the matching controls, but no statistically differences, whereas in group-B patients, the serum magnesium concentration is significantly higher than the healthy matching controls. The physical properties of the erythrocyte membrane are markedly affected by changes in cell Mg content.[35-36] The elevation or deficiency of magnesium level in SCP definitely has an important role in the nature of erythrocyte, but to the best of our knowledge there is very little investigative work done in this area. A few reports about abnormalities of Mg have been reported in children with ß–thalassemia.[37-38]

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