



Vitamin D, bilirubin and urinary albumin-creatinine ratio in adults with sickle cell anaemia

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Abstract

The interaction between vitamin D deficiency (VDD) and dysfunction of both the kidney and liver is still poorly understood in different health states of SCA. This study determined serum levels of vitamin D and indices of liver and renal function in adult sickle cell anaemia subjects. Sixty subjects with sickle cell anaemia (30 in steady state [SSCA] and 30 in vaso-occlusive crisis [VOC]) and 30 apparently healthy individuals with HbAA genotype were recruited into this study. Standard methods were used for the determination of total protein, albumin, bilirubin, urinary creatinine and albumin while serum vitamin D was determined using ELISA. Differences between groups were determined using Student's t-test or Man-Whitney U test as appropriate with $p < 0.05$ considered as statistically significant. Serum vitamin D was significantly lower in sickle cell anaemia (SCA) subjects and the deficiency was more profound in VOC when compared with the control subjects. SCA subjects with vitamin D level < 50 nmol/L had significantly higher levels of total bilirubin (TBIL) and conjugated bilirubin (CBIL) compared with those who had ≥ 50 nmol/L vitamin D level. There was no significant difference in vitamin D level between SCA subjects with one or less episode of SCA crisis per year and SCA subjects with two or more episodes of SCA crisis per year although, the median vitamin D level was lower in the latter. Vitamin D deficiency is more pronounced in SCA subjects in vaso-occlusive crisis and elevated serum bilirubin was observed in SCA subjects with low serum vitamin D level.

Keywords; Hyperbilirubinaemia, Sickle cell anaemia, Vaso-occlusive crisis, Vitamin D

INTRODUCTION

Sickle cell anaemia (SCA) is a common haemoglobinopathy affecting about 300,000 new births worldwide with Nigeria having the highest prevalence (WHO, 2006; Anie et al., 2010; Makani et al., 2011; Piel et al., 2013). Despite improved comprehensive care, the associated episodes of acute illness and progressive organ damage are still major health challenges in the management of SCA (Platt et al., 1994; Weatherall et al., 2005). These challenges are further compounded by the usually observed deficiency of vital nutrients such as vitamin D which has been associated with frequent acute pain episodes, chronic pain and musculoskeletal health in SCA patients (Osunkwo et al., 2011).

Previous reports showed that about 74% and 100% of children and adults with SCA respectively suffer from vitamin D deficiency (VDD) (Lal et al., 2006; Adewoye et al., 2008). Also, Mohammed et al. (1993) and Padmos et al. (1995) reported higher rates of VDD among African Americans with SCA. Despite these reports, VDD is still under-recognized and under-treated in individuals with SCA (Osunkwo et al., 2011).

One of the common complications, which are almost inevitable in SCA subjects, is renal damage. This has been attributed to sickle haemoglobin (HbS) polymerization

causing vaso-occlusion (due to erythrocyte dehydration) which results in renal infarction and medullary fibrosis with focal segmental glomerulosclerosis (Rees et al., 2010). Bolarinwa et al. (2012) reported that renal abnormalities are not uncommon in adult Nigerians with SCA. About 30% of adult SCA subjects develop chronic kidney disease, which is a contributory factor in many deaths (Platt et al., 1994).

The kidney is a major target organ for both the classical and non-classical actions of vitamin D (Li, 2010). Earlier reports showed that chronic kidney disease is associated with vitamin D insufficiency or deficiency (LaClair et al., 2005; Al-Badr and Martin, 2008).

One of the hall marks of nephropathy is albuminuria (Williams et al., 2009). An inverse relationship between the level of vitamin D and degree of albuminuria was reported from the Third National Health and Nutrition Examination Survey (NHANES III) (Williams et al., 2009; de Boer et al., 2007) suggesting that vitamin D have antiproteinuric effect (Li, 2010). These earlier reports indicate that optimal vitamin D status is highly important in slowing down renal damage in individuals with SCA.

Acute and chronic hepatic complications have been reported in individuals with SCA (Koskinas et al., 2007; Ebert et al., 2010). Hepatic problems are involved in about 39% of vaso-occlusive crisis (VOC) in SCA subjects (Ebert et al., 2010). Furthermore, 18% of young individuals with SCA were reported to suffer from liver cirrhosis with about 11% resulting in death (Darbari et al., 2006; Berry et al., 2007). This high rate of hepatic problems in SCA subjects has been attributed to hemosiderosis, hepatitis, zinc

deficiency and repeated clinically silent microvascular occlusions leading to liver fibrosis which is superimposed on other causes of chronic liver disease (Prasad et al., 1979; Koskinas et al., 2007).

Optimal liver function is crucial for vitamin D metabolism (hydroxylation of vitamin D to 25-hydroxyvitamin D) and bile acids are required for gastrointestinal absorption of vitamin D (Bickle, 2007; Holick, 2007). Thus, it is not surprising that VDD is a common observation in patients with liver disease (Nair, 2010; Stokes et al., 2013). The association between vitamin D status and liver diseases is a crucial one; low vitamin D may indicate liver dysfunction and VDD might contribute to liver damage through increased inflammation and fibrosis (Bikle, 2007; Bouillon et al., 2008; Pappa et al., 2008; Petta et al., 2010). Putz-Bankuti et al. (2012) reported a significant association between vitamin D level and the degree of liver dysfunction. Although there is clear evidence on association between vitamin D and liver disease, it is still unknown whether VDD confers an enhanced risk to liver disease or whether liver disease causes VDD (Stokes et al., 2013).

Due to the dearth of information on the interaction between vitamin D status and function of the kidney and the liver in individuals with SCA, this study determined serum levels of vitamin D and indices of liver and renal function in adult Nigerians with sickle cell anaemia.

MATERIALS AND METHODS

Subjects

Information on the study participants has earlier been reported (Akinlade et al., 2013). Briefly, 60 subjects with sickle cell anaemia (30 in steady state; SSCA and 30 in vaso-occlusive crisis; VOC) were recruited from the Haematology Day Care Unit, University College hospital, Ibadan while 30 apparently healthy individuals with HbAA genotype served as the controls.

Inclusion criteria

Absence of acute complicating factors or acute clinical symptoms or crisis for at least three months was used as indicator of steady state. This was established by a careful history and complete physical examination. However, presence of bone and joint pains or pain in multiple sites, requirement for analgesics and patients considering the episode as typical of crisis which necessitates hospital admission were clinically considered as indicators of VOC (Omoti et al., 2005).

Exclusion criteria

Subjects with HbAS (or other forms of genotype apart from HbSS and HbAA), diabetes mellitus, hypertension, human immunodeficiency virus (HIV), hepatitis, cancer and with established endocrine dysfunctions were excluded from this study. Lactating mothers, obese and subjects on anticonvulsants or glucocorticoids were also excluded from the study.

Ethical consideration

This study was approved by the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee. Also, written informed consent/assent

was obtained from each subject or his/her parent/guardian as appropriate.

Sample collection

About 8 ml of venous blood was obtained from each subject. The samples (4 ml each) were dispensed into plain and EDTA bottles to obtain serum and plasma respectively which were stored at -20°C until time of analysis. Also, 10 ml of random spot urine was collected into sterile universal bottle for urinalysis while aliquot of it was assayed for urinary creatinine and albumin.

Assay methodology

Genotype was determined using cellulose acetate electrophoresis at pH 8.6. Colorimetric method was used for the determination of total protein, albumin and bilirubin as described by Silverman and Christenson (1993), Dumas et al. (1971) and Jendrassik and Grof (1938) respectively. Serum vitamin D (25-hydroxyl vitamin D) was determined using ELISA (Immunodiagnostic Systems Ltd, UK) while urinary creatinine and albumin were determined using the Jaffe reaction and immunoturbidimetric (Elving et al., 1989) method respectively. Thereafter, SCA subjects were categorized into 2 groups based on their serum vitamin D level (<50 nmol/L and ≥50 nmol/L).

Statistical analysis

The distribution of the variables was assessed using histogram with standard curve. Total protein, albumin, total bilirubin (TBIL) and conjugated bilirubin (CBIL) (with Gaussian distribution) were compared between groups using the Student's t-test while vitamin D and urinary albumin creatinine ratio (UACR) (with non-Gaussian distribution) were compared using Man-Whitney *U* test. Statistical significance was set at $p < 0.05$. The SPSS statistical software program version 17.0 (SPSS Inc, Chicago, IL) was used for the statistical analysis.

RESULTS

In Table 1, a high proportion (90%) of the SCA subjects in steady state (SSCA) had insufficient vitamin D level with only 2 subjects having sufficient vitamin D level. Similarly, 63.3% and 36.7% of subjects in VOC had insufficient and deficient vitamin D status respectively. None of the VOC subjects had sufficient vitamin D level. Nine (30%) subjects with VOC and 6 (20%) subjects with SSCA had UACR ≥30 mg/g. However, proteinuria (using urine dip stick) was observed in only 5 subjects in VOC and 3 SSCA subjects.

In Table 2, the mean plasma levels of TBIL, CBIL and the median level of UACR were significantly higher while the median vitamin D level was significantly lower in the combined SCA (SSCA + VOC) group compared with the controls.

Comparing SSCA with controls, TBIL, CBIL and UACR were significantly higher while albumin was significantly lower. Similarly, TBIL, CBIL, and UACR were significantly higher while vitamin D was significantly lower in VOC subjects compared with controls. Only vitamin D was significantly lower in VOC subjects compared with SSCA (Table 3).

Table 1:
Vitamin D and renal status of the study participants

	SSCA (n = 30)	VOC (n = 30)	Controls (n = 28)
Vitamin D status			
Deficient (<25 nmol/L)	1 (3.3%)	11 (36.7%)	1 (3.6%)
Insufficient (25-74 nmol/L)	27 (90.0%)	19 (63.3%)	26 (92.9%)
Sufficient (75-250 nmol/L)	2 (6.7%)	0 (0.0%)	1 (3.6%)
Potential Intoxication (>250 nmol/L)	0 (0.0%)	0 (0.0%)	0 (0.0%)
UACR			
<30 mg/g	24 (80.0%)	21 (70.0%)	28 (100.0%)
≥30 mg/g	6 (20.0%)	9 (30.0%)	0 (0.0%)
Proteinuria (dip stick)	3 (10.0%)	5 (16.7%)	0 (0.0%)

Values are in number of participants (percentage)

Table 2:
Serum levels of vitamin D and indices of liver and renal status in Nigerians with sickle cell anaemia and controls

	Combined SCA (n = 60)	Controls (n = 28)	P-value
Age (years)	25.92±5.66	26.21±4.72	0.810
TBIL (mg/dl)	1.36±0.79	0.73±0.19	0.000*
CBIL (mg/dl)	0.41±0.28	0.27±0.09	0.014*
Total Protein (g/L)	69.05±6.50	69.86±7.71	0.611
Albumin (g/L)	40.20±4.25	42.00±4.75	0.078
UACR (mg/g)	15.25 (8.30-30.65)	5.35 (4.50-9.50)	0.000*
Vitamin D (nmol/L)	37.50 (28.50-48.88)	43.50 (38.13-57.00)	0.010*

*Significant at $P < 0.05$, values are in mean±standard deviation or median (interquartile range), TBIL=total bilirubin, CBIL=conjugated bilirubin, UACR=urinary albumin-creatinine ratio

Table 3:
Serum levels of vitamin D and indices of liver and renal status in SSCA, VOC and controls

	SSCA (n=30)	VOC (n=30)	Controls (n=28)
Age (years)	26.97±6.24	24.87±4.90	26.21±4.72
TBIL (mg/dl)	1.18±0.67 ^a	1.54±0.86 ^a	0.73±0.19
CBIL (mg/dl)	0.41±0.27 ^a	0.42±0.29 ^a	0.27±0.09
Total Protein (g/L)	67.30±4.68	70.80±7.60	69.86±7.71
Albumin (g/L)	38.77±4.14 ^a	41.63±3.92	42.00±4.75
UACR (mg/g)	14.10 (7.50-26.30) ^a	19.30 (8.98-36.95) ^a	5.35 (4.50-9.50)
Vitamin D (nmol/L)	43.50 (37.50-54.75)	30.50 (21.38-37.50) ^{a,b}	43.50 (38.13-57.00)

^aSignificantly different when compared with controls, ^bsignificantly different when compared with SSCA, values are in mean±standard deviation or median (interquartile range), TBIL=total bilirubin, CBIL=conjugated bilirubin, UACR=urinary albumin-creatinine ratio

Table 4:
Indices of liver and renal status in Nigerians with sickle cell anaemia based on vitamin D status

	<50 nmol/L (n = 45)	≥50 nmol/L (n = 15)	P-value
TBIL (mg/dl)	1.52±0.82	0.86±0.38	0.000*
CBIL (mg/dl)	0.45±0.31	0.28±0.08	0.001*
Total Protein (g/L)	69.58±6.80	67.47±5.38	0.280
Albumin (g/L)	40.56±4.10	39.13±4.64	0.265
UACR (mg/g)	16.30 (9.15-36.35)	15.20 (7.80-23.60)	0.249

*Significant at $P < 0.05$, values are in mean±standard deviation or median (interquartile range), TBIL=total bilirubin, CBIL=conjugated bilirubin, UACR=urinary albumin-creatinine ratio

Vitamin D level less than 50 to 75 nmol/L has been associated with increased parathyroid hormone (PTH) (Malabanan et al., 1998; Souberbielle et al., 2001) and reduced calcium absorption (Heaney et al., 2003) hence, the SCA subjects were categorized into 2 groups; <50 nmol/L and ≥50 nmol/L. It was observed that SCA subjects with vitamin D level <50 nmol/L had significantly higher levels of

TBIL and CBIL compared with those who had ≥50 nmol/L vitamin D level (Table 4).

In Table 5, the proportion of male (87.1%) subjects with SCA who had <50 nmol/L of vitamin D level was significantly higher than the proportion of female (62.1%) subjects with SCA who had <50 nmol/L of vitamin D.

Table 5:

Association of vitamin D status with age, gender, educational status, occupation and frequency of crisis per year in subjects with SCA.

	Vitamin D <50 nmol/L		n	X ²	P-value
	Yes (n=45)	No (n=15)			
Age (years)					
<25	22 (78.6%)	6 (21.4%)	28	0.357	0.550
≥25	23 (71.9%)	9 (28.1%)	32		
Gender					
Male	27 (87.1%)	4 (12.9%)	31	5.006	0.025*
Female	18 (62.1%)	11 (37.9%)	29		
Educational Status					
Primary & Secondary	9 (69.2%)	4 (30.8%)	13	0.295	0.587
Undergraduate & Graduate	36 (76.6%)	11 (23.4%)	47		
Occupation					
Skilled	3 (100.0%)	0 (0.0%)	3	1.353	0.508

Table 6:

Vitamin D status and biochemical indices of renal and liver function in SCA subjects based on frequency of crisis per year

	Once or less per year (n=26)	Two or more per year (n = 34)	P-value
Vitamin D (nmol/L)	40.75 (32.88-49.50)	34.00 (24.25-44.38)	0.181
UACR (mg/g)	14.60 (7.50-25.18)	15.80 (8.98-33.90)	0.507
TBIL (mg/dl)	1.25±0.65	1.44±0.88	0.372
CBIL (mg/dl)	0.36±0.19	0.45±0.33	0.206
Total protein (g/L)	69.08±6.51	69.03±6.59	0.978
Albumin (g/L)	38.65±3.92	41.38±4.16	0.012*

*Significant at $P < 0.05$, values are in mean±standard deviation or median (interquartile range), TBIL=total bilirubin, CBIL=conjugated bilirubin, UACR=urinary albumin-creatinine ratio

Mean serum albumin was significantly lower in SCA subjects with one or less episodes of SCA crisis per year compared with SCA subjects with two or more episodes of SCA crisis per year. There was no significant difference in vitamin D level between SCA subjects with one or less episode of SCA crisis per year and SCA subjects with two or more episodes of SCA crisis per year although, the median vitamin D level was lower in the latter (Table 6)

DISCUSSION

Vitamin D deficiency (VDD) and dysfunction of both the kidney and liver are common observations in individuals with SCA however; the interaction between them is still poorly understood. In this study, vitamin D level was significantly lower in SCA subjects compared with the controls. This observation is in line with earlier reports (Adewoye et al., 2008; Osunkwo et al., 2011). This is not unexpected as VDD is now considered as a key feature in SCA (Arlet et al., 2013). Lactose intolerance, poor sun exposure, hypermelanosis, hypermetabolic states, abnormalities of liver and kidney, impaired intestinal absorption of vitamin D and poor dietary vitamin D intake have been reported as some of the risk factors predisposing SCA subjects to VDD (Smiley et al., 2008). Also, the median vitamin D level was significantly lower in SCA subjects in VOC compared with the SSCA. This observation might indicate that there is a link between VDD and VOC hence; adequate vitamin D intake could be one of the therapeutic means of reducing episodes of VOC in SCA subjects. On the

other hand, the observed low vitamin D in VOC and SSCA could be as a result of illness itself which is usually characterized by increased inflammation. Autier et al. (2014) showed that VDD is not usually the cause of many diseases but the diseases themselves are responsible for the reported VDD. They also reported that inflammatory processes associated with disease have a negative effect on vitamin D levels. This explanation might hold for our observed low vitamin D in SSCA and more importantly, in VOC as we had earlier reported heightened systemic inflammation in this same group of SCA subjects in VOC (Akinlade et al., 2013). Further research would therefore be necessary to show if vitamin D supplementation has any benefit on reducing episodes of VOC or not.

We also observed that UACR was significantly higher in SCA subjects compared with controls. Similar results were also obtained when SSCA and VOC were compared with the control group. These observations further confirm the usual sickle cell nephropathy resulting from long-standing anemia and disturbed circulation through the renal medullary capillaries (Rees et al., 2010; Bolarinwa et al., 2012; Sasongko et al., 2013).

Hepatic problems are usual observations in SCA subjects and have been reported to be involved in VOC (Koskinas et al., 2007; Ebert et al., 2010). Not surprisingly, TBIL and CBIL were significantly higher in SCA subjects compared with the control subjects. Significant elevations were also observed in SSCA and VOC when compared with the controls. This observation could be due to the chronic haemolysis associated with SCA. Furthermore, the observed elevated CBIL could indicate impaired hepatic excretory function.

Our observed lower level of serum albumin in SSCA supports the report of Tripathi et al. (2011). Although the albumin level was within the reference range, our observation could indicate reduced synthesis of albumin in SCA since hepatic complications are commonly observed in this group of individuals. Furthermore, since SCA subjects usually experience heightened oxidative stress, the low albumin level could be due to oxidative modification which would lead to its rapid degradation. More so, it could be inflammation related (Kaul and Heibel, 2000) as albumin is a negative acute phase protein.

In order to understand the effects of vitamin D level on the biochemical parameters, SCA subjects were classified into 2 groups; <50 nmol/l and ≥50 nmol/l. It was observed that SCA subjects with vitamin D level of <50 nmol/l had higher TBIL and CBIL compared with SCA subjects who had higher levels of vitamin D. A similar observation was reported by Mutlu et al. (Mutlu et al., 2013). They associated low serum vitamin D level with hyperbilirubinaemia. Our observation could be as a result of possible impaired hepatic excretory function which could culminate in impaired bile acid formation causing impaired vitamin D absorption. Therefore, more research work is still required to delineate clearly which of the mechanisms of VDD is responsible for VDD in SCA subjects. This would improve the management of SCA subjects and improve their quality of life.

Considering the association of vitamin D status with gender, significant association was observed between male gender and low vitamin D status (<50 nmol/l). A similar gender discrepancy in serum vitamin D level was reported by Janz and Pearson (2013). They reported a higher blood level of vitamin D in females compared with males. Our observation probably suggests that there is optimal conservation of vitamin D in females than males however; more research work is required to proffer possible explanation to this observation.

Although vitamin D was lower in SCA subjects who experience 2 or more episodes of VOC per year than those with fewer VOC episodes, the difference was not statistically significant. However, albumin was significantly higher in SCA subjects who experience 2 or more episodes of VOC per year than those with fewer VOC episodes. This could be a result of dehydration as dehydration is one of the precipitating factors of VOC (Maakaron, 2013).

It could be concluded from this study that vitamin D deficiency is more pronounced in SCA subjects in vaso-occlusive crisis and elevated serum bilirubin was observed in SCA subjects with low serum vitamin D level.

Our study was limited by the small sample size and non-determination of the prevalence of lactose intolerance, evaluation of dietary intake of vitamin D or other lifestyle factors which could affect the development of VDD in this population.

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