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Spectrum of 25 (OH) D_3 Status in Urinary Tract Stone Formers in North-Eastern Nigeria

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ABSTRACT: Many studies, none in northeastern part of Nigeria, investigated the association between serum/plasma vitamin D and nephrolithiasis, with no consistent result, couple with the hot weather (as high as 44ºC) in most part of the year in Maiduguri and its surroundings, and assay of vitamin D today is not yet part of evaluation of urinary stone formers in Maiduguri, warrant this study to assess status of vitamin D in urinary tract stone formers in Maiduguri. Patients diagnosed with urinary tract stone disease attending Urology Clinic, University of Maiduguri Teaching Hospital and consented were recruited. The first two who consented were recruited per week for a period of 24 months (1st April 2017 to 31st Match 2019) to cover the seasonal changes in weather for the year. Serum for vitamin D is stored at -20ºC for batch analysis. Vitamin D was assayed by ELISA technique (Accu-Bind, 100 North Pointe Drive, Lake Forest, California 92630 USA), other parameters were analyzed weekly using autoanalyzer (Cobas C311, ISN, Roche, Germany). Result indicated 58 (36.2%) of patients had Vitamin D values below the optimal level either having deficiency/insufficiency. Forty-nine (30.6%) showed vitamin D concentration above the optimum level out of which 4 (2.5%) had hypervitaminosis D, while 53 (33.1%) patients had optimal vitamin D values. It showed 17 (10.6%) of patients had hypercalcaemia, however, out of the 114 patients with normocalcaemia, 42 (36.5%) had their values at the upper limit of normal (2.6 mmol/L). It also indicated that though 3 of the hypercalcaemic patients had vitamin D insufficiency, majority (14) are either having optimal level or above optimal vitamin D level. Excessive and prolong exposure to ultraviolet sunlight resulting in raised vitamin D synthesis and action is an important factor associated with urinary tract stone formation in this environment cannot be excluded. The high prevalence of hypercalcaemia in the study cannot be accounted for by raised vitamin D alone, therefore measurement of serum vitamin D (25(OH)D) and PTH in evaluation of urinary tract stone formers in this environment is recommended.

KEYWORDS: Maiduguri, North-eastern Nigeria, Urinary tract stone disease, Vitamin D_3 status.

INTRODUCTION

Urinary tract stone disease is common with an estimated prevalence of about 10-15% in males and 3-5% in females in the general population,[1] a lifetime risk of about 11% for men and 7% for women, and tends to increase with changes in diet and climate globally.[2] An incidence of 32/100,000 was reported some time ago in Maiduguri,[3] and was found to be higher than in other parts of Nigeria, Lagos,[4,5] Zaria and Nwewi.[6] It is a recurrent disease with a recurrence rate as high as 50% at five years and 80%-90% at 10 years[7] particularly if the primary cause is not sought and treated. Apart from economic burden, urolithiasis may result in life-threatening conditions, such as decrease renal function, hydronephrosis, pyonephrosis, and may even result in end-stage renal failure.[8,9] The current methods of management of renal stone in the study area are focus mainly on the stone itself rather than 'stone disease'. Therefore, further investigations of nephrolithiasis are high priorities for the development and improvement of medical therapy and prevention.

Calcium is the most frequent component and major constituent, 75%-80%, of urinary tract stones. [10] Hypercalciuria is the commonest metabolic abnormality identified in calcium stone formers, [10] demonstrated in up to 30%-60% of adults with urinary tract stone in Maiduguri, [11] high urine calcium excretion is a strong risk factor for stone formation. [1]

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Vitamin D, a necessary hormone and nutrient for human, is the key regulator of calcium and phosphorus metabolism. One of a major source of vitamin D is irradiation of skin by ultra-violate light and in the study area, most part of the year has high ambient temperature with clear sky uncovered by cloud and also majority of the population practice either farming or cattle raring, it is expected that this will facilitates increase vitamin D synthesis and might be a reason for higher incidence of stone disease in the study environment.

Many studies, none in northeastern part of Nigeria, investigated the association between serum/plasma vitamin D and nephrolithiasis, however, their results are not consistent,[12, 13] couple with the hot weather (as high as 44ºC) condition in most part of the year in Maiduguri and its surroundings, and assay of vitamin D today is not part of evaluation of urinary stone formers in Maiduguri. Therefore, this warrant this study to assess status of vitamin D in urinary tract stone formers in Maiduguri.

SUBJECTS AND METHODS

Scope of the study

The study was limited to patients diagnosed with urinary tract stone disease attending urology unit of University of Maiduguri Teaching Hospital, Maiduguri.

Methodology

Study area (www.bornostate.gov.org)

The study was conducted over a period of 24 months. Borno State, north-eastern Nigeria, is located on Latitude 11.41 N and Longitude 13.16 E with an annual rainfall between 500 to 900 mm/year in the Sahel Savannah region of the country. Temperature, as shown in figure 1, can be as high as 44ºC in the most part of the year, generally ranges between 31ºC and 44ºC

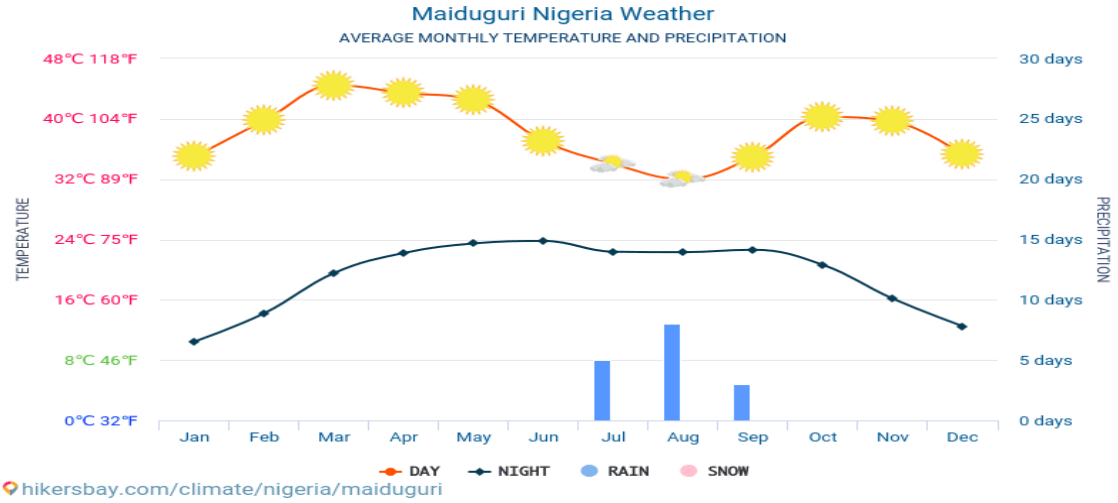

Figure 1: Average monthly temperature and precipitation in Maiduguri, Nigeria (www.bornostate.gov.org)

STUDY DESIGN: Prospectus cross-sectional observational study

Sample size, sample collection and sample analyses. Samples were collected from patients who are diagnosed with urinary tract stone disease attending Urology Clinic, University of Maiduguri Teaching Hospital and consented to participate in the study. Subsequently, we decided to recruit two patients per week for a period of 24 months (1st April 2017 to 31st Match 2019) to cover the seasonal changes in weather for the year. We recruited the first two who consented for the study for each week. After collecting biodata from patients, 10ml venous blood was collected from the antecubital vein aseptically and allowed to clot at room temperature. Sample was centrifuged at 5000 revolutions per minute (rpm) for 10 minutes. Serum for vitamin D is stored at -20ºC for batch analysis. Analyses for other parameters were done weekly.

PROCEDURES:

SAMPLE ANALYSES: Total protein was analyzed using Biuret method,[14] Albumin by colorimetric Bromocresol green method[15] while uric acid was analyzed by enzymatic colorimetric method[16], total calcium by colorimetric (o-cresolphalein

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complexone method[17] and in-organic phosphate by Molybdate U.V. method[18] .These are done using the autoanalyzer (Cobas C311, ISN, Roche, Germany) in the Department of Chemical pathology, UMTH, while vitamin D was assayed by ELISA technique (Accu-Bind, 100 North Pointe Drive, Lake Forest, California 92630 USA)[19]

STATISTICAL ANALYSIS: The data generated in this study were analyzed using statistical package for social sciences (SPSS) version 18.0 for windows. We used the manufacturer's instruction to classify patients into various spectra consequent to different cut-off values and scanty studies concerning vitamin D3 in study environment. We interpret the rest of the parameters (corrected plasma total calcium, inorganic phosphate, uric acid, urea, creatinine, and albumin) using departmental reference values to sought out those with abnormalities and biochemical evidence of associated renal insufficiency among patients.

RESULTS

One hundred and sixty (160) urinary tract stone disease patients were recruited for the study over a period of 24 months (1st April 2017 to 31st Match 2019). Of these 66.3% were males and 28.1% were females with a male: female ratio of about 2.5:1. However, gender was omitted for 5.6% of patient, as indicated in figure 2 and table 1

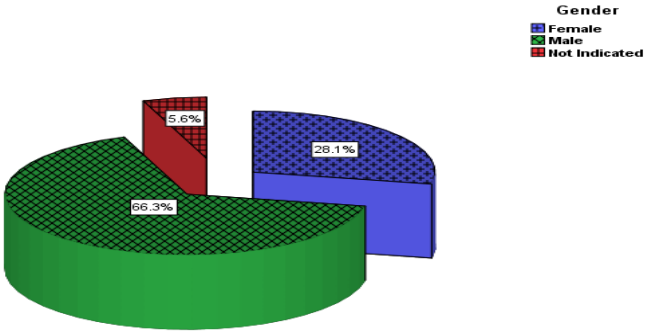

Figure 2: Gender Distribution among the Participants of the Study.

Figure 3 and table 1 showed distribution of patients by age. These demonstrated many of the urinary tract stones formers are in Paediatric age range with 32(20%) of patients were 19 years and below. A substantial number of patients are within the age range 20 and below 60 years and (34.4%) were between 20-50 years. The youngest was 2 years old while the oldest was 80 year old. There was no record of age of 39 (24.4%) of total number of patients. This is as depicted in fig 3

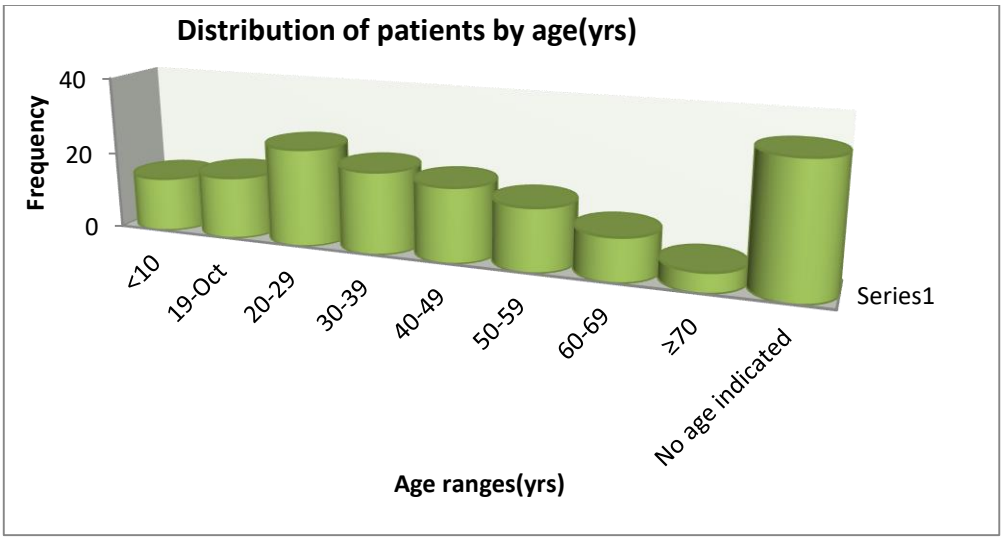

Figure 3: Frequency distribution of patients by age (yrs)

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Table 1: Demographic Characteristics of the Participants of the Study.

Variables	Frequency (N)	Percentage (%)
Gender		
Female	45	28.1
Male	106	66.3
Not indicated	9	5.6
Total	**160**	**100.0**
Age (years)		
< 10	17	10.6
10-19	15	9.4
20-29	24	15.0
30-39	15	9.4
40-49	16	10.0
50-59	17	10.6
60-69	11	6.9
≥ 70	6	3.8
Not indicated	39	24.4
Total	**160**	**100.0**

Many literatures reported values ranging from 30ng/ml to as high as up to 70ng/ml as adequate (accepted upper limit for different populations). Also due to scanty studies on vitamin D in study environment, we adopted the classification of vitamin D spectrum provided by the manufacturer and as shown in table 2 below

Table 2: Classification of vitamin D status by values (based on manufacturer's instruction)

Vitamin D values(ng/ml)	Status
≤20ng/ml	Vitamin D deficiency
21-30ng/ml	Sub-optimal(Insufficiency)
31-50ng/ml	Optimal
51-70ng/ml	Upper normal
71-150ng/ml	Over-dose but not toxic
>150ng/ml	Hypervitaminosis

Table 2 shows categorization of patients based on their serum vitamin D values, indicating that 58 (36.2%) of patients had values below the optimal level either having deficiency/insufficiency. Forty-nine (30.6%) showed vitamin D concentration above the optimum level out of which 4 (2.5%) had hypervitaminosis D. While 53 (33.1%) patients had optimal vitamin D values

Table 3: Distributions of patients according to vitamin D status (Vitamin D Spectrum in patients)

Vitamin D_3(status)	***Number of patients***	***Percentage***
Deficiency	29	18.1
Sub-optimal(insufficiency)	29	18.1
Optimal	53	33.1
Upper Normal	16	10.0
Overdose but not Toxic	29	18.1
Hypervitaminosis	4	2.5
Total	**160**	**100.0**

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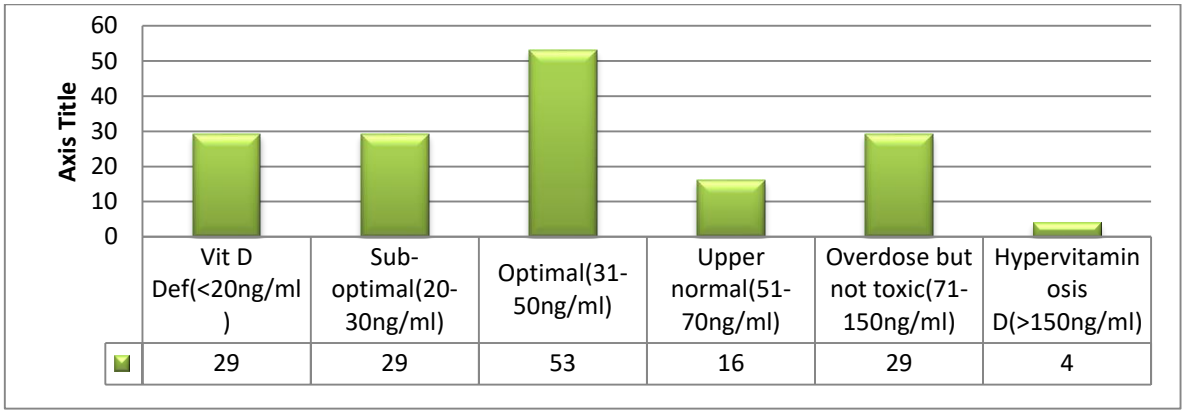

Figure 4: Spectral distributions of patient according to their respective serum vitamin D levels

Table 4 depicted other analytes analysed in patients' serum during the study. It showed 17 (10.6%) of patients had hypercalcaemia, however, out of the 114 patients with normocalcaemia, 42 (36.5%) had their values at the upper limit of normal (2.6 mmol/L)

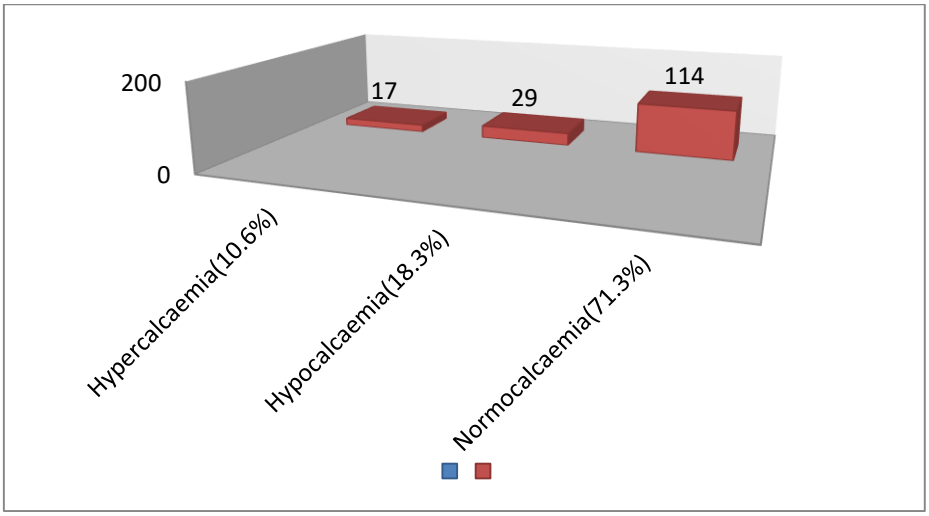

Figure 5: Distribution of patients by plasma total calcium concentration

Of the 160 patients, 74 (46.3%) had raised plasma inorganic phosphate values and only 35 (21.9%) had hyperuricaemia. Out of the 17 patients who had hypercalcaemia, 11 also had Hyperphosphataemia, similarly 28 of those with normocalcaemia also had hyperphosphataemia

Forty-two patients had increased plasma urea level where as 29 of them had their values below 10 mmol/L and 13 with values equal to or greater than 10 mmol/L, out of which 12 had mild to moderate increased plasma creatinine. Twenty-nine patients had mild to moderate increased plasma creatinine with no record of 12 patients. Of the 160 patients, 38 (23.8%) are hypoalbuminaemic and only 1 (0.6%) patient is hyperalbuminaemic

Table 4: Shows Levels of Calcium, Albumin, Inorganic Phosphate, Uric Acid, Urea and Creatinine in the Participants of the Study.

Variables	Frequency (N)	Prevalence (%)
Calcium		
Normocalcaemia	114	71.3
Hypocalcaemia	29	18.1
Hypercalcaemia	17	10.6
Total	**160**	**100.0**

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Albumin		
Normoalbuminaemia	121	75.6
Hypoalbuminaemia	38	23.8
Hyperalbuminaemia	1	0.6
Total	**160**	**100.0**
Inorganic Phosphate		
Normal	79	49.4
Hypophosphataemia	1	0.6
Hyperphosphataemia	74	46.3
Not Indicated	6	3.8
Total	**160**	**100.0**
Uric Acid		
Normal	113	70.6
Hyperuricaemia	35	21.9
Not Indicated	12	7.5
Total	**160**	**100.0**
Urea		
Normal	106	66.3
Increased(azotaemia)	42	26.3
Not Indicated	12	7.5
Total	**160**	**100.0**
Creatinine		
Normal	119	74.4
Increased	29	18.1
Not Indicated	12	7.5
Total	**160**	**100.0**

Table 5 showed plasma total calcium distribution with respects to spectral distribution of vitamin D. It indicated that though 3 of the hypercalcaemic patients had vitamin D insufficiency, majority (14) are either having optimal level or above optimal vitamin D level

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Table 5: Levels of Calcium and Vitamin D3 in the Participants of the Study.

Variables	***Calcium***			
	Normocalcaemia	***Hypocalcaemia***	***Hypercalcaemia***	**Total**
Vitamin D₃				
Deficiency	19	9	1	29
Sub-optimal	22	5	2	29
Optimal	37	11	5	53
Upper Normal	10	2	4	16
Overdose not Toxic	25	2	2	29
Hypervitaminosis	1	0	3	4
Total	**114 (71.3)**	**29 (18.1)**	**17 (10.6)**	**160 (100.0)**

Table 6 showed that plasma albumin distribution has no influence on spectral distribution on plasma vitamin D despite its role as a carrier protein, only one patient had hyperalbuminaemia

Table 6: Levels of Albumin and Vitamin D_3 in the Participants of the Study.

Variables	***Albumin***			
	Normoalbuminaemia	***Hypoalbuminaemia***	***Hyperalbuminaemia***	**Total**
Vitamin D₃				
Deficiency	22	7	0	29
Sub-optimal	20	9	0	29
Optimal	43	10	0	53
Upper Normal	8	8	0	16
Overdose, not Toxic	27	2	0	29
Hypervitaminosis	1	2	1	4
Total	**121 (75.6)**	**38 (23.8)**	**1 (0.6)**	**160 (100.0)**

DISCUSSION

Male preponderance and a male: female ratio 2.5:1 noted and concurs with many studies elsewhere, presenting male preponderance and male: female ratios ranging from 1.35:1 in Nepal [20] to 4:1 in Benin, [21] Nigeria. A decreasing ratio was noted in Taiwan [22] and was thought to be due to increasing obesity, diabetes mellitus and metabolic syndrome among their women

Twenty percent (20%) of the participants were 19 years and below including a 2 old child. This demonstrated the need to investigate not only the stone itself but the stone disease otherwise such a child has high rate of recurrence with aging. Fifty-five (34.4%) of the patients were within the age range 20-50 years which is the most productive age group. This is similar to findings in other studies in Nigeria,[23,24] Ghana,[25] Saudi Arabia,[26] Nepal,[20]and Taiwan[22] recording peak age range from 20-40 years.

The likely potential factors predisposing children/adolescents having stone disease in this environment may include: (a) It was found in one study that children/adolescents from the Middle East/African regions had significantly higher vitamin D values than in North America and Europe and this could be explained by the fact that children/adolescents from this region generally spend more time outdoors compared with the other age groups;[27] (b) children/adolescent in Africa, in particular, spend much of their time hawking causing them to sweat a lot. It is known that minimal fluid intake, resulting in decreased urine production and a high concentration of stone-forming salts in urine, are leading factors in renal calculi development. Decreased fluid intake and urine production was noted to be the most common predisposing factor in 45.3% of patients studied in Ghana.[25] Other factors that we may be dealing with are: (c) inhibited activity of 24-hydroxylase or elevated activity of 1α-hydoxylase, both leading to increased concentration of the active vitamin D metabolite,[28] which was not measured in the study, and normal or even decreased 25(OH) D_3,[29] which was decreased in many of the patients studied; (d) Increased expression of VDRs or the saturation of the capacity of

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VDBP.[30] (e) Another recently discovered cause of idiopathic infantile hypercalcaemia(IIH) involves a defect in SLC34A1, the gene coding for the sodium-phosphate cotransporter(NaPi-IIH) in the kidney.[31] Hypercalcaemia is the indirect manifestation of the downregulation of FGF-23,[32] (f) Xanthine and Cysteine constituted 1.7% and 0.9%, respectively, of stone composition we analysed in our earlier study in this environment which mainly present in childhood and may still account for the number of children found in this study. A family history of kidney stone disease is one of the strongest causes of penetration for kidney stone formation. The overall impact of genetic disorders contributing to the development of nephrolithiasis in patients is astonishingly high. Roughly 10% of individuals affected by nephrolithiasis can attribute some aspect of their condition directly to heritable disease. [33] An autosomal recessive genetic disease Cystineuria is directly responsible for 1% of all kidney stones. [34] These diseases lead to the development of cysteine stone, which are highly infectious and affect people of all ages. This disorder is commonly found in children and account for roughly 25% of childhood nephrolithiasis. [35]

In this study, 36.3% had 25(OH)D_3 below optimal (vitamin deficiency/insufficiency), 28.1% had 25(OH)D_3 above the optimal level and 2.5% with hypervitaminosis D, and only 33.3% had optimal concentration of 25(OH)D_3. A systematic review of vitamin status of population worldwide [27] found that 88.1% of the results reviewed had mean 25(OH)D_3 values below 30ng/ml, 37.3% had mean values below 20ng/ml and 6.7% had mean values below 10ng/ml, indicating that vitamin D deficiency is common worldwide and as noted even among stone formers in this environment which may similarly reflect the status of vitamin D in this environment.

In a study by Perez-Barrios, *et al.,*[36] hypervitaminosis D was found in 1.86%) of which 11.1% displayed hypercalcaemia. In this study 2.5% patients found with hypervitaminosis D and all had hypercalcaemia.

In his 1999 review, Vieth[37] noted that serum 25(OH)D levels above 80ng/ml are not rare among healthy persons with ample sun exposure. This is also found in 33(20.6%) of the study population. Farming, hawking and cattle raring are common in this environment and may explain the high number of patients with 25(OH)D above optimal values. Vieth[37] also noted that of the 30 outdoor workers in whom they measured 25(OH)D in late summer, 3 (10%) had levels above 80ng/ml compared to the 33(20.6%) we found in our study indicating possible increased vitamin D synthesis in this environment. Less than 10% of vitamin D is derived from dietary sources in the absence of food fortification or use of supplements, and about 80% is synthetic via skin exposure to ultraviolet light. The likely link between high vitamin D and urinary tract stone are increase intestinal calcium absorption. This was noted in a study that intestinal calcium transport increased by 45% to 65% in women when 25(OH) D levels were increased from 20 to 32ng/ml.[38] It is known also that 25(OH) D levels are inversely associated with parathyroid hormone (PTH) levels until the former reach 30 to 40 ng/ml, at which point parathyroid hormone levels begin to level off (at their nadir).[39] Increased intestinal calcium absorption associated with suppressed parathyroid result into hypercalciuria together with profuse sweating subject to harsh weather and decreased urine output may encourages urinary tract stone formation. Parathyroid hormone however was not assayed in this study. This may explain why 10.6% of the study patients presented with hypercalcaemia, the maximum were 2.9mmol/L, and however 36.5% have plasma calcium values at the upper limit of normal (2.6mmol/L). As stated above, the presence of serum vitamin D above the optimal level facilitates intestinal calcium absorption, the resultant suppressed parathyroid hormone increases urinary calcium excretion (hypercalciuria) maintaining the plasma calcium levels within reference limit but the associated hypercalciuria will encourages urinary stone formation.

The biochemical difference between increased PTH and that of increased vitamin D functions are that in hypervitaminosis D both plasma calcium and inorganic phosphate are raised because vitamin D encourages intestinal absorption of both calcium and inorganic phosphate and similarly increases renal reabsorption of both calcium and inorganic phosphate, mainly secondary to suppressed PTH while a raised plasma calcium and decreased plasma inorganic phosphate is found in increased PTH functions.

In the study population, 74 (46.3%) had raised plasma inorganic phosphate values and only 35 (21.9%) had hyperuricaemia stressing that the raised plasma inorganic phosphate cannot be accounted for by renal retention. Usually both uric acid and inorganic phosphate are proportionately increased in renal insufficiency as retention products of insufficient glomerular filtration. [40]. In this study however, in contrast, only 21.9% presented with hyperuricaemia. Similarly, 17 patients who had hypercalcaemia, 11 of them also had Hyperphosphataemia, and 28 of those with normocalcaemia also had Hyperphosphataemia. The Hyperphosphataemia is likely to be accounted for by raised plasma vitamin D and not renal insufficiency in the study population. More so, although 42 (26.3%) of the patients had increased plasma urea, 29 (18.1%) had values less than 10 mmol/L and 13 (8.1%) with values equal to or greater than 10 mmol/L, of these 12 (7.5%) had mild to moderate increased plasma creatinine much less than the 46.3% of those who had raised plasma inorganic phosphate. Twenty-nine patients had mild to moderate increased plasma creatinine. Therefore, the

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Hyperphosphataemia noted in the study cannot be accounted for solely by renal insufficiency, but likely due to the raised vitamin D in some of the patients in the study population

The study also demonstrated that although 6 of the hypercalcaemic patients had vitamin D insufficiency, majority (11) either have optimal level or above optimal vitamin D level and is likely due to increased action of vitamin D_3. Plasma parathyroid hormone, a possible cause of hypercalcaemia, was not measured in the study.

Conclusion: Excessive and prolong exposure to ultraviolet sunlight resulting in raised vitamin D synthesis and action is an important factor associated with urinary tract stone formation in this environment cannot be excluded. The high prevalence of hypercalcaemia in the study cannot be accounted for by raised vitamin D alone, therefore measurement of serum vitamin D (25(OH)D) and PTH in evaluation of urinary tract stone formers in this environment is recommended.

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CONFLICT OF INTEREST

There was no conflict of interest.

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