ORIGINAL RESEARCH

Vitamin D Levels and Insulin Resistance among Nigerian men with Type-2 Diabetes mellitus

Abbiyesuku FM[1], Olawale OO*[2], Agbakwuru AN[3], Olooto WE[2]

[1]Department of Chemical Pathology, University of Ibadan, Ibadan, Oyo state, Nigeria.
[2]Department of Chemical Pathology and Immunology, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Sagamu, Ogun state, Nigeria.
[3]Department of Chemical Pathology, National Orthopaedic Hospital, Igbobi, Lagos State, Nigeria.

Abstract

Background: A number of studies have shown a high prevalence of insufficient vitamin D levels in humans in the North American, European and Asian regions. Various research works have also shown that low serum vitamin D levels play a major role in the pathogenesis of chronic, non-infective illnesses such as diabetes mellitus and cancer.

Objective: This study was aimed at assessing the serum vitamin D status in relation to glucose homeostasis among men with Type-2 Diabetes mellitus and normal controls.

Methods: This comparative cross-sectional study included 80 men with confirmed diagnosis of Type-2 diabetes mellitus and 49 normal adult male controls. Serum 25-hydroxy vitamin D, fasting serum C-peptide and fasting plasma glucose levels were measured in both study groups.

Results: There was a significant difference between the mean serum 25-OH vitamin D levels among the cases (36.55ng/mL) and the controls (42.96ng/mL) (p = 0.001). All the four 25-OH vitamin D-deficient subjects had diabetes. In the diabetes group, 43.8% had a normal insulin resistance compared to 61.8% of the control group (p = 0.054). In the diabetes group, 73.8% had sufficient vitamin D, 21.2% had insufficient vitamin D and 5% had vitamin D deficiency. In the control group, there was a significant negative correlation between serum 25-OH vitamin D and BMI and fasting plasma glucose. The mean HOMA2IR value for the diabetes group (3.09) was higher than the value for the controls (2.40).

Conclusion: The mean serum 25-OH vitamin D level in the diabetes group was lower than that of the control group hence, hypovitaminosis D may be a contributor to the onset of diabetes mellitus among Nigerian men.

Keywords: C-peptide, Diabetes mellitus, HOMA, Insulin resistance, Vitamin D.

Introduction

Diabetes mellitus (DM) comprises a group of common metabolic disorders that share the phenotype of hyperglycaemia.

*
Correspondence
Department of Chemical Pathology and Immunology, Olabisi Onabanjo University, Sagamu, Ogun state, Nigeria.
Telephone: 08025891259
Email: bosundipupo@yahoo.com

Many distinct types of DM exist and are caused by a complex interaction of genetics, lifestyle choices and environmental factors.[] Depending on the aetiology of DM, factors contributing to hyperglycaemia may include reduced insulin secretion, decreased glucose utilisation and increased glucose production.[] DM is a serious international public health issue, affecting more than 180 million people worldwide.[]

A study in Port Harcourt, Nigeria gave a crude prevalence rate of DM of 6.8%. [] The high DM burden in Africa can be attributed to westernisation of lifestyles and inadequate physical activity. Type-2 diabetes mellitus is the most common type of the disease in clinical practice. The pathogenesis of Type-2 DM remains obscure but insulin resistance, impaired beta cell function and systemic inflammation have been implicated. More recent studies suggested that vitamin D also influences these pathways. [] The present treatment modalities in DM include the use of oral hypoglycaemic agents (OHA), insulin therapy and lifestyle modifications. However, recent research, focus on the various preventive measures in DM and these are aimed to reduce hospital and clinic attendance as well as the expenditure on drug procurement. []

Vitamin D is a hormone that is known to be involved in calcium homeostasis and bone formation. [] However, recent research showed that vitamin D also play important roles in cancer prevention, immunity and glucose homeostasis. Humans get vitamin D through exposure to sunlight, from diet and from dietary supplements. [] High prevalence of vitamin D insufficiency has been observed worldwide and these have occurred concurrently with insulin resistance, which in turn, is implicated in the aetiology of Type 2 DM. [] Factors that contribute to vitamin D deficiency include increased age, decreased exposure to sunlight, increased skin pigmentation and obesity. [] If it can be established that vitamin D deficiency contributes to the onset of Type-2 diabetes mellitus in Nigerians, the information will open a new frontier in the management of DM. Vitamin D deficiency is easily correctable through dietary supplementation and more exposure to the ultraviolet B rays of the sun.

The objective of the study was to evaluate the prevalence of Vitamin D insufficiency/ deficiency among men with Type-2 DM in comparison with men without diabetes. The study can serve as a prelude to bigger studies to evaluate the relevance of vitamin D supplementation in the prevention and treatment of DM.

Methods

Study design
This was a comparative cross-sectional study of serum vitamin D and C-peptide levels among adult males with Type-2 DM who were on treatment with oral hypoglycaemic drugs (OHA). The serum vitamin D, C-peptide and fasting plasma glucose levels were measured and compared between the two groups of subjects. The study included 80 adult men with confirmed Type-2 diabetes mellitus attending University College Hospital, Ibadan while 49 apparently healthy men residing in Ibadan and its environment served as controls. The respective cases and controls were matched for social class. All the participants in the study were recruited after granting written informed consent.

Sample size determination
The minimum sample size required to detect a difference in mean 25-OH Vitamin D between subjects with Type-2 DM and healthy controls was derived using the formula:
$$n = [2(Z\alpha + Z1\text{-}\beta) 2\sigma^2] / (\mu_1 - \mu_2)^2$$
Where $Z\alpha$ = standard normal deviate corresponding to 5% level of significance = 1.96
$Z1\text{-}\beta$ = standard normal deviate corresponding to a power of 90% = 1.28
σ = standard deviation of 25 (OH) Vitamin D = 23 nmol/L (Targher *et al* 2006). [12]
$\mu1 - \mu2$ = the minimum difference in mean 25 (OH) Vitamin D between subjects with diabetes and control groups which is assumed to be clinically significant = 15
n = minimum sample size in the two groups.
Although a minimum of 49 subjects per group was required, a non-response rate of 15% was assumed thus, 58 subjects were studied per group.

Study site and Ethical considerations
The study was conducted at the Metabolic Research Unit of the University College Hospital Ibadan, Oyo state, Nigeria. Ethical approval for the study was granted on the 17th March 2011 by the University of Ibadan/ University College Hospital (UI/UCH) Health Research Ethics Committee with number EC/10/0199.

Inclusion and Exclusion criteria
Adult men with confirmed diagnosis of Type-2 DM attending the Metabolic Research Unit (MRU)

clinic were recruited into the study. Subjects with acute or chronic illnesses (except Type-2 DM and hypertension) and those who withheld consent were excluded from the study. Subjects with plasma creatinine levels ≥ 1.5mg/dl were also excluded from the study. Male members of staff of the University College Hospital without DM were recruited as controls. The controls with impaired fasting plasma glucose (5.6-6.9mmol/L[100-125mg/dL]) were excluded from the data analysis at the final stage. Only respondents with fasting plasma glucose <5.6mmol/L (100mg/dL) were included in the control group.

Data Collection
Information on demographic characteristics and lifestyle of subjects were obtained using a semi-structured pre-tested questionnaire.
Anthropometric measurements were taken using standard procedures. A calibrated double beam balance was used to measure weight in kilograms (kg) with the subjects in very light clothing. The height in metres (m) was measured using a stadiometer. The measurements were taken by only one assessor to remove the inter-observer error.

Patient preparation
All the subjects had overnight fast lasting 8-12 hours. In addition, drugs which may affect the glucose flux (e.g. steroids) were avoided prior to the fast. All the subjects in the case group took their oral hypoglycaemic drugs (OHA) as prescribed by their physicians.

Sample collection and storage
The serum measurements were determined thus: 8mls of venous blood was aseptically obtained from a vein in the antecubital fossa with minimal stasis using pyrogen-free disposable needles and syringes. Serum for 25-OH vitamin D and the C-peptide assay was obtained by dispensing 5mLs of the venous blood into plain vacutainer tubes and allowing to clot and retract for 30 minutes, following which it was centrifuged and stored at -20°C. The serum C-peptide assay was done using the enzyme-linked immunosorbent assay (ELISA) method. 25-OH vitamin D level was also determined using the high performance liquid chromatography (HPLC) technique. The remaining 3ml venous blood was placed in Fluoride vacutainer tubes for glucose assay using the enzymatic technique. The plasma was separated within one hour and stored at -20°C until the final assay was done.

Quality Control
The serum samples and standards were run in duplicate. High and low controls were added at the beginning, middle and end of the assay run.

C-peptide Assay
Serum C-peptide levels were assayed using the solid phase enzyme-linked immunosorbent assay (ELISA) kit (cat # 1293Z) from Diagnostic Automation, Inc. ® Calabasas, California 91302, USA. (www.rapidtest.com). The kit had an intra-assay and inter-assay Co-efficient of Variation (CV) of 5.9% and 8.28% respectively. The kit had a detection range of 0.5-20ng/mL and an analytical sensitivity of 0.1ng/mL. The assay results were read off using a Thermo-Fisher Multiskan EX microplate reader at a wavelength of 450nm according to the manufacturer's protocol.

Glucose Assay
Previously frozen plasma was used for glucose assay. Glucose estimation was done using the Randox Glucose® (GLUC-PAP) kit manufactured by Randox Laboratories Ltd., Ardmore, United Kingdom. The analytic machine used was the Perlong PU2018G semi-automated chemistry analyser. Randox multisera controls at 2 levels were used to assess precision and accuracy. Inter-assay CV was 6%. The test protocol was as stated by the manufacturer.

Vitamin D Assay
Total serum 25-OH vitamin D was assayed using the Waters 616 HPLC® machine manufactured by the Waters Corporation USA. Serum samples previously stored at -20°C were used for the assayed within 3 months. Serum vitamin D status was categorized into three groups namely: vitamin D deficient (< 20ng/mL), vitamin D insufficient (20 - 29.99ng/mL) and vitamin D sufficient (≥ 30ng/mL). [9,11]

Insulin resistance

The HOMA2 (Homeostasis Model Assessment2) calculator software was used to estimate insulin resistance using fasting plasma glucose and C-peptide levels. The HOMA calculator uses model-derived analysis to determine β cell function, insulin sensitivity and insulin resistance in the steady state condition. [13] HOMA has been used in over 500 studies and the validity of this method was reviewed by Wallace *et al*. [14] The insulin resistance threshold used in the study was 2.77 according to the results of a study conducted by Borona *et al* [15] in 1998.

Data analysis

The Statistical Package for Social Sciences (SPSS) version 16.0 was used for data processing. Socio-demographic and other measured variables were summarized depending on the variable type using means and standard deviation. The mean values of measured variables were compared between groups using Student's t-test. Cross-tabulation was used to test the proportional relationship between Vitamin D and insulin resistance within each study group. The correlation between Vitamin D levels and other measured variables were tested using a correlation analysis within groups. The Pearson's correlation analysis was used for normally distributed data and Spearman's correlation for skewed data. The level of statistical significance for all tests was 95%.

Results

Unit conversions for measured analytes were as follows:
1nmol/L = 1ng/mL x 2.5   (25-OH vitamin D)
1nmol/L = 1ng/mL x 0.33   (C-peptide)
1mmol/L = 1mg/dL x 0.056   (Glucose)

The mean 25-OH vitamin D values for the diabetes group (36.5ng/mL) was significantly lower than that of controls (42.96ng/mL) (p = 0.006). The mean fasting plasma glucose level was significantly higher among the subjects with diabetes (8.45mmol/L [152.1mg/dL]) than among the controls (5.0mmol/L [90.14mg/dL]) (p

< 0.001).

The mean BMI of the cases (24.88kg/m^2) was similar to that of the controls (24.59kg/m^2) (p = 0.740). The mean HOMA2IR value for the cases (3.09) was significantly higher than for the controls (2.40) (p = 0.655). The mean serum C-peptide value of the cases (3.56ng/mL) was slightly lower than that of the controls (3.26 ng/mL) (p = 0.376). A comparison of the data for both study groups is depicted in Tables I and II.

Table I: Comparison of mean values of the physical characteristics in both groups

Variables	Cases n = 80 Mean(SD)	Controls n = 49 Mean (SD)	P-values
Age (years)	61.66 (10.4)	54.50 (11.1)	<0.001
Body Mass Index (BMI) (kg/m2)	24.59 (5.5)	24.88 (3.5)	0.740
Height (m)	1.7017 (0.07)	1.71 (0.06)	0.437
Weight (kg)	73.35 (13.1)	72.92 (13.2)	0.856

Almost all the cases (92.5%) took metformin/glibenclamide, 5% took only metformin while 1.2% was on pioglitazone/metformin. Most of the cases (68.7%) did not have co-morbidities while 31.3% had hypertension with diabetes.

Table II: Comparison of the mean values of the measured analytes in both groups

Variables	Cases n = 80 Mean (SD)	Controls n = 49 Mean (SD)	P-values
C-Peptide (ng/mL)	3.56 (1.69)	3.26 (2.07)	0.376
Vitamin D (ng/mL)	36.55 (11.3)	42.96 (10.1)	0.001
Fasting Plasma Glucose (mmol/L)	8.45 (3.87)	5.0 (0.49)	<0.001
HOMA2IR	3.09 (1.4)	2.40 (1.5)	0.014
HOMA2 %Insulin Secretion	42.423 (28.4)	66.35 (51.2)	<0.001
HOMA2 % Beta Cell Function	111.4 (97.6)	167.5 (73.4)	0.004

The study groups were subdivided according to age intervals of 25 years i.e. 30 - 55 years (younger age group) and 56 - 80 years (older age group). This is to adjust for the impact of age on the measured variables since the mean age was significantly different between both study groups. The cases had lower levels of mean serum 25-OH vitamin D

compared to the controls in both age groups. In the 30 - 55 years group, the cases had mean serum vitamin D of 39.4±15.4ng/mL compared to 44.2±11.9ng/mL (p = 0.219) in the control group. In the 56 - 80 years group, the cases had mean serum vitamin D levels of 44.2±11.9ng/mL compared to 41.4±7.3ng/mL in the control group (p = 0.004). The mean values of all the other variables (insulin resistance, fasting plasma glucose, C-peptide and body mass index) were higher among the cases for both age groups as shown in Table III.

Table IV shows the cross-tabulation of serum 25-OH vitamin D levels and insulin resistance for both study groups. All four 25-OH vitamin D-deficient subjects had diabetes. In the group with diabetes, 43.8% had a normal insulin resistance compared to 61.8% of the control group (p = 0.054). In the diabetes group, 73.8% had sufficient vitamin D, 21.2% had insufficient vitamin D, 5% had vitamin D deficiency. Ninety percent of the controls had sufficient vitamin D levels while only 10% had insufficient vitamin D levels.

Table III: Comparison of measured parameters in both groups based on age groups

	30-55 years			56-80 years		
	Cases	Control	P-values	Cases	Control	P-values
n	22	27		58	22	
C-peptide ng/ml	3.3 (1.7)	3.1 (1.9)	0.750	3.7 (1.7)	3.4 (2.3)	0.653
Vit D ng/ml	39.4 (15.4)	44.2 (11.9)	0.219	35.5 (9.3)	41.4 (7.3)	0.004
BMI Kg/m²	26.1 (4.2)	24.1 (2.6)	0.043*	24.9 (3.7)	25.9 (4.2)	0.359
IR	2.9 (1.3)	2.3 (1.4)	0.128	3.2 (1.5)	2.5 (1.7)	0.136
FPG (mg/dl)	9.2 (4.1)	5.0 (0.46)	<0.001*	8.2 (3.8)	5.0 (0.54)	<0.001

n = number, Vit D = 25 - OH vitamin D, BMI = Body mass index, IR = Insulin resistance, FPG = Fasting plasma glucose

Table IV: Cross-tabulation of vitamin D status and insulin resistance in each study group

		Insulin resistance				Total	
		Normal (≤2.77)		Raised (>2.77)			
		Diabetic	Control	Diabetic	Control	Diabetic	Control
Vitamin D deficiency	Count	2	0	2	0	4	0
	% Vitamin D status	50.0%	0%	50.0%	0%	100.0%	0%
	% Insulin resistance	5.7%	0%	4.4%	0%	5.0%	0%
Vitamin D insufficiency	Count	10	3	7	4	17	7
	% Vitamin D status	58.8%	42.9%	41.2%	57.1%	100.0%	100%
	% Insulin resistance	28.6%	10%	15.6%	21.1%	21.2%	14.3%
Vitamin D sufficiency	Count	23	27	36	15	59	42
	% Vitamin D status	39.0%	64.3%	61.0%	35.7%	100.0%	100%
	% Insulin resistance	65.7%	90.0%	80.0%	78.9%	73.8%	85.7%
Total	Count	35	30	45	19	80	49
	% Vitamin D status	43.8%	61.2%	56.2%	38.8%	100.0%	100%
	% Insulin resistance	100.0%	100%	100.0%	100%	100.0%	100%
	% of Total	43.8%	61.2%	56.2%	38.8%	100.0%	100%

Discussion

The present study showed a significant difference between mean serum 25-OH vitamin D levels of 36.55ng/mL in the diabetes group and 42.96ng/mL in the control group. This supports the findings in many previous studies. Hypponen et al reported lower levels of serum vitamin D in people with Type-2 diabetes (15ng/mL) compared to the controls (21ng/mL) in the 1958 British Birth Cohort Survey. [15] Cigolini et al, in 2006, also reported a similar result of 20ng/mL among people with diabetes compared to 24ng/mL among the controls. [16] It is believed that adequate vitamin D is required for the secretion of insulin by the β cells of the pancreas due to the crucial role calcium plays in this process.

Interestingly, all the four individuals with vitamin D deficiency in the present study had diabetes. This observation suggests a very high degree of positive correlation between vitamin D deficiency and diabetes mellitus. However, due to the small number of individuals within this sub-group in the present study, generalization may be difficult thus, a larger study is required to validate this theory. On the other hand, only 14.3% of the controls in the present study had 25-OH vitamin D insufficiency compared to 26.2% of the diabetes group.

The mean BMI was similar in both diabetes and control groups thus making the comparison between the two study groups stronger. The members of each study group were further subdivided into two age groups (35-55 and 56-80)

with age intervals of 25 years each. The reason for this was to adjust for the influence of age on the measured parameters. The mean serum vitamin D level was lower in the diabetes group for each age sub-group compared to the control group. This shows that even after adjusting for the significant age difference between the two study groups, mean serum vitamin D was still significantly higher in the control group. The present study also found reduced levels of vitamin D in the older age group compared to the younger age group. This observation supports the reports of previous studies that vitamin levels decrease gradually with age. [17]

There appears to be a variation in the serum vitamin D levels in African studies. Ethiopian men had mean serum vitamin D level of 9.4ng/mL (23.5nmol/L),[] in Zairian men the mean value was 26ng/mL (65nmol/L),[] while among Fulani men, the serum vitamin D level ranged from 10ng/mL to 30ng/mL (25-75nmol/L). [16] It is, however, difficult to compare serum 25-OH vitamin D values from different studies because of difference in the analytic methods employed to assay the 25-OH vitamin D levels and the lack of an internationally standardized assay. [19]

The mean serum C-peptide level was not significantly different between the diabetes group (3.56 ± 1.69ng/mL) and the controls (3.26 ± 2.07ng/mL). It is expected that mean serum C-peptide levels will be higher in the diabetes group because of increased insulin secretion to overcome insulin resistance. However, later in life, people with diabetes may have decreased serum C-peptide levels because of exhaustion of β cell reserves. Therefore, serum C-peptide values in individuals with Type-2 diabetes may be raised or depressed depending on the state of the β cells.

The mean insulin resistance in the diabetes group was significantly lower than the value in the control group in the present study. The insulin resistance values from this study were also much lower than the results of the study reported by Oli *et al* in Enugu, in which people with diabetes had 9.4 ± 12.9 while the controls had 3.4 ± 2.8. However, both studies supported the finding that insulin resistance was significantly higher in the diabetes groups. The reason for this large difference between the studies may be due to the fact that the original HOMA equation was used for the Enugu study while the present study used the updated HOMA2 algorithm which puts renal glucose

loss and changes in hepatic and peripheral glucose resistance into consideration. [20] In addition, fasting serum insulin was used in the Enugu study while fasting serum C-peptide was used in this study. The use of HOMA to make comparisons across ethnic groups is valid, but the baseline HOMA-%S from a normoglycaemic population in each comparative group should be established first in order to determine whether a difference in insulin sensitivity between groups implies a different baseline. [20]

In the diabetes group, there was a significant negative correlation between the logarithm of serum C-peptide and the logarithm of fasting plasma glucose. 25-OH vitamin D had no significant correlation with fasting serum C-peptide, insulin resistance or body mass index but there was a weak negative correlation with fasting plasma glucose. This finding was similar to the reports made by Orwoll *et al* [21] in 1994 and Scragg in 2004 [17] where correlations were found in Hispanics and non-Hispanic whites but not in blacks. No explanation could be given for the lack of correlation in blacks but Orwoll proposed that blacks may exhibit a different vitamin D homeostasis compared to the other ethnic groups. There may actually be a genetic difference in vitamin D homeostasis between ethnic groups but this can only be proven by further molecular studies. Larger sample sizes in future studies may also give more power to the study and may show associations that are not evident in this study.

Conclusion

The present study showed that serum 25-OH vitamin D less than or equal to 20ng/mL (50nmol/L) was associated with Type-2 diabetes mellitus. The mean serum 25-OH vitamin D level in the diabetes group was significantly lower than that of the control group hence it is likely that hypovitaminosis D may be a contributor to the onset of diabetes mellitus. A larger cohort study designed to find the effect of vitamin D supplementation on diabetes mellitus will help to further investigate this theory. It is also important to standardize serum 25-OH vitamin D assay in order to facilitate the comparison of data across centres. No association was found between serum 25-OH vitamin D levels and insulin resistance in the present study. Molecular studies are required to investigate the association between serum

vitamin D level, insulin resistance and diabetes mellitus in the sub-Saharan population.

References

1. Powers AC. Diabetes Mellitus. In: Kasper DL, Fauci ASB, E. Longo DL, Jameson JL, Hauser SL (Editors). Harrison's Principles of Internal Medicine. 16th Edition: McGraw-Hill; Chicago. 2005: 2152.

2. Mokdad AH, Bowman BA, Ford ES, Vinicor F, Marks JS, Koplan JP. The continuing epidemics of obesity and diabetes in the United States. JAMA 2001; 286(10): 1195-1200.

3. Nyenwe EA, Odia OJ, Ihekwaba AE, Ojule A, Babatunde S. Type 2 diabetes in adult Nigerians: a study of its prevalence and risk factors in Port Harcourt, Nigeria. Diabetes Res Clin Pract 2003; 62(3): 177-185.

4. Alfonso B, Liao E, Busta A, Poretsky L. Vitamin D in diabetes mellitus-a new field of knowledge poised for the development. Diabetes Metab Res Rev 2009; 25(5): 417-419.

5. Pittas AG, Dawson-Hughes B. Vitamin D and diabetes. J Steroid Biochem Mol Biol 2010; 18: 425-429.

6. Lips P, Bouillon R, van Schoor NM, Vanderschueren D, Verschueren S, Kuchuk N, et al. Reducing fracture risk with calcium and vitamin D. Clin Endocrinol (Oxf) 2009; 10.

7. Holick MF. Vitamin D: extraskeletal health. Endocrinol Metab Clin North Am 2010; 39(2): 381-400.

8. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. Am J Clin Nutr 2004; 79(5): 820-825.

9. Holick MF. Vitamin D Deficiency. New Eng J Med [Review] 2007; 357: 266-281.

10. Webb AR, Holick MF. The role of sunlight in the cutaneous production of vitamin D3. Annu Rev Nutr 1988; 8: 375-399.

11. Lips P. Worldwide status of vitamin D nutrition. J Steroid Biochem Mol Biol 2010; 121(1-2): 297-300.

12. Targher G, Bertolini L, Padovani R, Zenari L, Scala L, Cigolini M, et al. Serum 25-hydroxyvitamin D3 concentrations and carotid artery intima-media thickness among type 2 diabetic patients. Clin Endocrinol (Oxf) 2006; 65(5): 593-597.

13. Diabetes TOcf. HOMA CALCULATOR. 2010 [cited 2010]; Available from: http://www.dtu.ox.ac.uk/homacalculator/index.php.

14. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care 2004 ; 27(6): 1487-1495.

15. Hypponen E, Power C. Vitamin D status and glucose homeostasis in the 1958 British birth cohort: the role of obesity. Diabetes Care 2006; 29(10): 2244-2246.

16. Cigolini M, Iagulli MP, Miconi V, Galiotto M, Lombardi S, Targher G. Serum 25-hydroxyvitamin D3 concentrations and prevalence of cardiovascular disease among Type 2 diabetic patients. Diabetes Care 2006; 29(3): 722-724.

17. Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. Diabetes Care 2004; 27(12): 2813-2818.

18. Glew RH, Crossey MJ, Polanams J, Okolie HI, VanderJagt DJ. Vitamin D status of seminomadic Fulani men and women. J Natl Med Assoc 2010 ; 102(6): 485-490.

19. Lips P, Duong T, Oleksik A, Black D, Cummings S, Cox D, et al. A global study of vitamin D status and parathyroid function in postmenopausal women with osteoporosis: baseline data from the multiple outcomes of raloxifene evaluation clinical trial. J Clin Endocrinol Metab 2001; 86(3): 1212-1221.

20. Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Targher G, et al. Prevalence of insulin resistance in metabolic disorders: the Bruneck Study. Diabetes 1998; 47(10): 1643-1649.

21. Orwoll E, Riddle M, Prince M. Effects of vitamin D on insulin and glucagon secretion in non-insulin-dependent diabetes mellitus. Am J Clin Nutr 1994; 59(5): 1083-1087.