Diurnal Variation of Serum 25 (OH) D_3, Calcium and Phosphorus in Type 2 Diabetes Mellitus

[1]Rupendra Shakya , [1]Balram Neupane , [1]Devi Prasad Bhandari

[1]Faculty of Health Science, Crimson College of Technology and Crimson Hospital, Butwal-11, Nepal

ABSTRACT

Diurnal variation, an outgrowth of chronobiology, is the inferential statistical mapping of structures in variables; in and around us, consisting of rhythms chaos and trends. Type-2 diabetes mellitus is a metabolic disorder with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. The diurnal time structure of serum 25(OH) D_3 and calcium may relate to the prevention and chronotherapeutic efficacy and management of type 2 diabetes mellitus. To our knowledge, the diurnal variation of serum 25(OH) D_3 has not yet been reported in type 2 diabetic patients. The present study was planned to evaluate the diurnal variation of serum 25(OH) D_3, calcium and phosphorus levels in type 2 diabetic patients. Ten clinically healthy volunteers and ten diagnosed patients of type 2 diabetes mellitus of similar age groups were synchronized for one month with diurnal activity from about 06:00 to about 22:00 and nocturnal rest. All subjects took their meals three times daily without any change in their usual fluid intake. Blood sample were collected into plain and sterile vials under quality control procedures from each participant at every 6 hour. Serum 25(OH) D_3, serum Ca^{++}, serum PO_4, FBS and PPBS levels were estimated. A marked diurnal variation in serum 25(OH) D_3 was recorded in healthy subjects (P=0.030). Similarly, a circadian rhythm of borderline statistical significance was also recorded for vitamin D in diabetic patients (P=0.083) and in healthy participants for serum calcium (P=0.070), phosphorus (P=0.102), and the calcium-phosphorus ratio (P=0.091) by the Two way ANOVA analysis. In addition, the amplitude and acrophase differed from healthy participants in diabetic patients for studied variables with a change of MESOR for calcium-phosphorus ratio. Mapping the broader time structure of different physiological variables investigated herein may be helpful in understanding the treatment and prevention of diabetic mellitus.

Key words: Serum 25(OH) D_3, Calcium, Phosphorous, Type 2 Diabetes mellitus

Corresponding address: Rupendra Shakya, Department of Bsc.MLT, Crimson College of Technology and Crimson Hospital Affiliated to Pokhara University,
E-mail:shakyarupen80@gmail.com

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.[1] Diabetes is a chronic condition associated with serious morbidity increased mortality and is rapidly becoming a global epidemic.[2] Vitamin D is a fat-soluble vitamin that is naturally present in very few foods, and is also produced endogenously when ultraviolet rays from sun strike vitamin D synthesis.[3] It was estimated that 1 billion individual presented vitamin D deficiency or insufficiency.[4] Vitamin D promotes calcium absorption in the gut and maintains adequate serum calcium and phosphate concentrations to enable normal mineralization of bone and to prevent hypocalcemic tetany. It is also needed for bone growth and bone remodeling by osteoblasts and osteoclasts.[5,6] Much evidence suggested that vitamin D is involved in several mechanisms in addition to bone metabolism[7] and its role in abnormal glucose metabolism as well as in type 2 diabetes has been demonstrated.[8,9] A recent review indicated that vitamin D deficiency may predispose to glucose intolerance, altered insulin secretion and type 2 diabetes[10] either through a direct action via vitamin D receptor activation or indirectly through calcemic hormones and also by inflammation.[11] Interest in Vitamin D and Type-2 diabetes mellitus was stimulated by early animal studies identifying a Vitamin D receptor in pancreatic tissue[12] and data showing that Vitamin D deficiency affected insulin secretion.[13] Only a very few studies have established regarding the diurnal variation of serum 25 (OH) D_3 in healthy individuals and other conditions and the role of vitamin D in abnormal glucose metabolism as well as in type 2 diabetes has been demonstrated. Hence, we evaluated the diurnal variation of serum 25 (OH) D_3 with serum Ca^{++} and PO_4 among type-2 diabetic patients as compared with healthy volunteers.

MATERIALS AND METHODS

The present study was conducted at the Crimson Hospital and Lumbini Medical College & Teaching Hospital, Department of Biochemistry and was planned as a prospective cross-sectional trial. It included subjects suffering from Type-2 diabetes

mellitus who attended at the out-patient and in-patient in the department of medicine between December 2017 to January 2018. Out of 20 patients, 10 were allocated as a study group and 10 in the control group. They were investigated by the respective consultants as per the diabetic clinic protocol and additional investigations were done as and when required. They followed a 24-hour synchronized social schedule with a diurnal activity from about 06:00 to about 22:00 hours and nocturnal rest and subjects slept from 10:00 to 06:00h.All subjects took their (although not identical) meals three times daily; breakfast around 8:30 a.m., lunch around 13:30 and dinner around 20:30 p.m., without any change in their usual fluid intake. 5 ml of blood was collected from each subject at 6 hourly intervals at fixed points for one complete 24-hour span i.e. at 06:00, 12:00, 18:00 and 00:00 hour in plane vials. The relevant tests were done from Central laboratory of Crimson Hospital. The blood tests included as, the serum 25(OH) D_3, serum Ca^{++}, serum Po4, fasting and PPBS were estimated. The data obtained was coded and were analyzed by Two-way Analysis of Variance in Microsoft Excel.

RESULTS AND OBSERVATIONS

Mean serum 25(OH) D_3 levels, were found to be maximum at 12:00 with a value of 13.30±2.62 (ng/ml), which diminished markedly during the rest of the day reaching a minimum at 06:00 in healthy controls. Similarly, maximum serum 25 (OH) D_3 was observed at 12:00 which declined during the rest of the period reaching minimum at 06:00 with a mean of 10.13±3.51 (ng/ml) in diabetic patients. These differences were significant at studied clock hours (P<0.05).

Table 1 : Two-way ANOVA of serum 25 (OH) D_3 (ng/ml) levels in age-matched healthy volunteers and diabetic patients at different collection hours of a 24-hour light-dark period.

Source of Variance	Normals		Patients	
	Among times	Among Normals	Among times	Among Patients
Degree of freedom (DF)	3	9	3	9
Sum of Squares (SS)	43.21	169.43	14.61	374.40
Mean Squares (MS)	14.40	18.82	4.87	41.60
F	2.66	3.48	3.38	28.88
P	>0.06	<0.05	>0.05	<0.05

At the sampling hours of 06:00 a.m, 12:00p.m, 18:00p.m and 00:00a.m ; the mean serum 25(OH) D_3 levels were 10.68, 13.31, 11.52, 10.86, in healthy controls and 10.13, 11.72, 10.61 and 10.39 in diabetic patients respectively.

Mean serum Ca^{++} levels, found to be maximum at 12:00p.m with a value of 8.95±1.23 (mg/dl), which decreased markedly during the rest of the day reaching a minimum at 00:00a.m in healthy controls. These variations at different collection hours during a 24 hours cycle were not significant (P>0.05). The maximum serum Ca^{++} was observed at 18:00p.m which decreased during the rest of the period reaching a minimum at 06:00a.m with a mean of 7.87±1.35 (mg/dl) in diabetic patients. These differences were not significant at studied clock hours (P>0.05).

Table 2 : Two-way ANOVA of Serum Ca^{++} (mg/dl) levels in age-matched healthy volunteers and diabetic patients at different collection hours of a 24-hour light-dark period.

Source of Variance	Normals		Patients	
	Among Times	Among Normals	Among Times	Among Patients
Degree of freedom (DF)	3	9	3	9
Sum of Squares (SS)	5.01	61.65	4.81	20.29
Mean Squares (MS)	1.67	6.85	1.60	2.25
F	0.69	2.83	1.82	2.56
P	>0.05	<0.05	>0.05	<0.05

At the sampling hours of 06:00a.m, 12:00p.m, 18:00p.m and 00:00a.m; the mean serum Ca^{++} levels were 8.38, 8.95, 8.20, 8.00, in healthy controls and 7.87, 8.39, 8.75, and 8.68 in diabetic patients respectively.

Mean serum Po4 levels, found to be maximum at 00:00a.m with a value of 5.01±1.19 (mg/dl), which decreased markedly during the rest of the day reaching a minimum at 18:00 in healthy controls. These variations at different collection hours during a 24 hours cycle were not significant (P>0.05). The maximum serum Po4 was observed at 18:00 which decreased during the rest of the period reaching a minimum at 06:00 with a mean of 4.17±1.40 (mg/dl) in diabetic patients. These differences were not significant at studied clock hours (P>0.05).

Table 3 : Two way ANOVA of serum Po4 (mg/dl) levels in age-matched healthy volunteers and diabetic patients at different collection hours of 24-hour light dark period.

Source of Variance	Normals		Diabetic Patients	
	Among Times	Among Normals	Among Times	Among Patients
Degree of freedom (DF)	3	9	3	9
Sum of Squares (SS)	2.93	18.65	1.28	97.55
Mean Squares (MS)	0.97	2.07	0.42	10.83
F	0.64	1.36	0.80	20.42
P	>0.05	>0.05	>0.05	<0.001

At the sampling hours of 06:00a.m, 12:00p.m, 18:00p.m and 00:00a.m; the mean serum Po4 levels were 4.96, 4.86, 4.33, 5.01 in healthy controls and 4.17, 4.36, 4.65, and 4.52 in diabetic patients respectively.

Mean serum Ca^{++}: Po4 levels, found to be maximum at 12:00p.m with a value of 2.04±0.59, which decreased markedly during the rest of the day reaching a minimum at 00:00a.m in healthy controls. These variations at different collection hours during a 24 hours cycle were not significant (P>0.05). The maximum serum Ca^{++}: Po4 was observed at 00:00a.m which decreased during the rest of the period reaching a minimum at 12:00p.m with a mean of 2.02±0.44 in diabetic patients. These differences were not significant at studied clock hours (P>0.05).

Table 4 : Two way ANOVA of serum Ca^{++}: Po4 levels in age-matched healthy volunteers and diabetic patients at different collection hours of a 24-hour light-dark period.

Source of Variance	Normals		Diabetic Patients	
	Among Times	Among Normals	Among Times	Among diabetic
Degree of freedom (DF)	3	9	3	9
Sum of Squares (SS)	1.03	3.27	0.04	7.20
Mean Squares (MS)	0.34	0.36	0.01	0.80
F	2.10	2.22	0.14	8.31
P	>0.05	<0.05	>0.05	<0.001

At the sampling hours of 06:00a.m, 12:00p.m, 18:00p.m and 00:00a.m; the mean serum Ca^{++}: Po4 levels were 1.72, 2.04, 1.90, 1.63, in healthy controls and 2.03, 2.02, 2.07, and 2.10 in diabetic patients respectively.

Mean serum Ca++× Po4 Product levels were found to be maximum at 12:00p.m with a value of 42.56±13.38 (mg^2/dl^2), which declined gradually during the rest of the period reaching a minimum at 18:00 with a value of 36.15 in healthy subjects. These variations at different collection hours during a 24 hours cycle were not significant (P>0.05). The maximum serum Ca++× Po4 Product was observed at 18:00 which declined during the rest of the period reaching a minimum at 06:00a.m with a mean of 33.19±13.54 (mg^2/dl^2) in diabetic patients. These differences were not significant at studied clock hours.

Table 5 : Two way ANOVA of Serum Ca^{++} × Po4 Product (mg^2/dl^2) levels in age-matched healthy volunteers and diabetic patients at different collection hours of a 24-hour light-dark period.

Source of Variance	Normals		Diabetic Patients	
	Among Times	Among Normals	Among Normals	Among Patients
Degree of freedom(DF)	3	9	3	9
Sum of Squares (SS)	269.80	3712.65	565.93	11434.72
Mean Squares (MS)	89.93	412.51	188.64	1270.52
F	0.49	2.25	1.42	9.60
P	>0.05	<0.05	>0.05	<0.001

At the sampling hours of 06:00a.m, 12:00p.m, 18:00p.m and 00:00a.m; the mean serum Ca^{++}×Po4 Product levels were 42.20, 42.56, 36.15, 41.48 in healthy controls and 33.19, 36.70, 43.30, and 40.01 in diabetic patients respectively.

DISCUSSION AND CONCLUSION

The results of the present study demonstrated a statistically significant diurnal variation in serum 25 (OH)D_3 in type 2 diabetic patients (P<0.05).The serum 25 (OH) D_3 was noticed to be maximum at 12:00 noon with a mean of 11.72±3.53 and a minimum at 06:00 a.m with a mean of 10.13±3.51 in type 2 diabetic patients. Only a few investigators have studied whether total plasma levels of serum 25(OH)2 D3 vary in a diurnal pattern. Lars Rejnmark et al. (2002) studied that plasma levels of vitamin-D binding protein (DBP) and total 1, 25(OH)2 D3 vary in a statistically significant diurnal rhythm. In a group of postmenopausal women,a significant diurnal pattern of variation in plasma concentrations serum 25(OH)2 D3 and DBP was found, but not in the calculated free serum 25(OH)2 D3 index. Thus, the variations were most likely caused by diurnal changes in plasma volume[14]. On the other hand, there was no diurnal variation of serum 25 (OH) D_3 in healthy volunteers (P>0.05). Mean serum 25(OH) D_3 levels found to be maximum at 12:00 with a value of 13.30±2.62 (ng/ml), which diminished markedly during the rest of the day reaching a minimum at 06:00a.m in healthy controls. These variations at different collection hours during a 24 hours cycle were not significant (P>0.05). In healthy volunteers, P value (0.06) found in our study is quite close to be significant as compared to the standard P value (<0.05). There was no diurnal variation in serum 25 (OH) D_3 in healthy volunteers (P>0.05) Besides, we measured serum Ca^{++}, Po4, Ca^{++}: Po4 and Ca^{++} × Po4 product every 6 hours interval for 24 hours in type-2 diabetic patients and also in healthy volunteers. There was no diurnal variation in serum Ca^{++}, Po4, Ca^{++}: Po4 and Ca^{++} × Po4 product in type 2 diabetic patients and in healthy volunteers during 24 hours cycle (P>0.05). Likewise, Prince et al. did not found any significant variation in plasma concentrations of serum 25(OH)2 D3 as a function of time when examined between 08:00 and 22:00h [15]. Similarly, Adams et al.[16] and Halloran et al.[17] reported that the plasma levels of serum 25(OH)2 D3, in eleven young males and females (aged 19–48 years) and five young males (aged 21–40 years) respectively, were maintained within relatively narrow limits when measured for a 24-h period. Similarly, Juttmann J R et al. (1981) were measured serum concentrations of 25-hydroxy cholecalciferol (25-OH2D), 24, 25-dihydroxy cholecalciferol (24, 25-(OH)2 D) and 1, 25-dihydroxy cholecalciferol (1, 25-(OH) 2 D3) at monthly intervals throughout the year in eight normal subjects[18]. A seasonal variation, apparently dependent on exposure to ultraviolet light, was found for all three metabolites. A study in six other normal subjects showed that there was no diurnal rhythm in any of the metabolites. The amazing result was found in the study done by William Jubiz et al.[19] in 1972 they determined a circadian variation in serum calcium and phosphorous concentration in normal subjects. The serum calcium levels were highest at 8:00 p.m. and reached a nadir between 2:00 and 4:00 a.m.

Another study done by Ridefelt P et al. (2012) demonstrated a diurnal variation of total calcium in serum during night-time and day-time conditions in seven healthy volunteers. Their study showed mean calcium values slightly higher in the afternoon in comparison with samples collected in the morning[20]. The no diurnal variation might be the reason for decrease in blood calcium, phosphorous and increased blood glucose in type 2 diabetic patients. Consequently, the diurnal variation in the level of serum 25 (OH)D_3 could be the reason for the problem of inadequate release of insulin in type-2 diabetic patients and might be the cause of insulin resistance and increased blood glucose levels in type-2 diabetic patients. In clinical studies, the diurnal variations of serum 25(OH)D_3 in type-2 diabetics must be considered and the anti-diabetic therapy can be modified according to the biological clock to deal more effectively with increased blood glucose levels and for better therapeutic utilization and efficiency. Furthermore, the extensive study can be carried out to reflect more on the relation of type 2 diabetes mellitus with 25 (OH) D_3.

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