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Determination of Serum 25 (OH) D Concentration in All Study Subjects

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Abstract **Original Research Article**

Background: Various studies reported that Low serum vitamin D has been found to be associated with various types of metabolic illness such as obesity, diabetes mellitus, insulin resistance, cardiovascular diseases including hypertension. Low serum 25 (OH) D levels have been linked to a range of non-skeletal health conditions in adults, including metabolic disorders and cardiovascular diseases. ***Objective:*** To determine serum 25 (OH) D concentrations in all study subjects. ***Methods:*** This cross-sectional analytical study was carried out to determine serum 25(OH)D concentration in all study subjects. For this study, 90 subjects were recruited after fulfilling the inclusion and exclusion criteria. Study subjects were divided into two groups: 45 subjects with MetS and 45 subjects without MetS. ***Results:*** Mean±SD of Age (years), Height (m), Weight (kg), BMI (kg/m2), WC (cm), SBP (mmHg), DBP (mmHg) in Group I and Group II. Subjects with MetS had higher Weight (kg), BMI (kg/m2), WC (cm), SBP (mmHg), DBP (mmHg) than those subjects without MetS. The relationship of BMI, FPG with Vitamin D became significant when FPG entered into model 2. Again, it was evident that Vitamin D was associated with height, BMI and FPG and these relationships remained significant even after TG and HDL-C entered into model 3. ***Conclusion:*** In conclusion we can say that low serum vitamin D concentration may predispose to higher BMI, WC, SBP and FPG also low serum vitamin D concentration is negatively correlated with components of MetS (WC, SBP and FPG).

Keywords: Metabolic syndrome, vitamin D concentrations, fasting plasma glucose.

INTRODUCTION

Low serum 25 (OH) D levels have been linked to a range of non-skeletal health conditions in adults, including metabolic disorders and cardiovascular diseases. Epidemiological studies show that serum concentrations of vitamin D are inversely associated with MetS [1].

Vitamin D deficiency has been identified as a public health issue [1]. Vitamin D deficiency reduces the intracellular calcium levels and thereby depletes insulin secretion by β-cells, which further impairs glucose tolerance [2]. Vitamin D deficiency could be a risk factor for MetS [3, 4].

Vitamin D is a fat-soluble vitamin that may be trapped and saved in adipose tissue. Studies reported more storage of Vitamin D in adipose tissue and lower bioavailability of endogenous Vitamin D in blood circulation of obese persons [5] Serum 25-hydroxyvitamin D concentrations are low in obese adults (Zamboni *et al.*, 1988; Yanoff *et al.*, 2006) and linked to components of body composition, particularly body fat mass (Liel *et al.*, 1988; Danescu, Levy & Levy, 2009) [6-9]. Supplementations with vitamin D have shown a positive outcome in reducing body fat mass in women regardless of their body mass index (BMI) levels [10].

Vitamin D is thought to play a protective role against the development of type-2 diabetes by improving the insulin secretion of pancreatic beta cells and by maintaining glucose homeostasis [11, 12]. Higher vitamin D levels might have a protective effect against the development of MetS [13, 14]. Vitamin D supplementation has shown a significant drop in

abdominal visceral adipose tissue in obese and overweight adults [15].

Vitamin D supplementation might improve ovulatory dysfunction and thereby revives fertility in women with polycystic ovarian syndrome [16, 17]. Vitamin D administration inhibited secretion of parathyroid hormone [18].

The major circulating form of vitamin D is 25-hydroxyvitamin D [25(OH)D], which has a half-life of approximately 2-3 weeks [19]. Thus, measurement of 25(OH)D is the accepted indicator of an individual's vitamin D status [20]. However, $1,25(OH)_2D$ (calcitriol) has a half-life of only four to eight hours. therefore, measurement of circulating $1,25(OH)_2D$ does not provide a useful assessment of an individual's vitamin D status as vitamin D deficiency leads to parathyroid hormone (PTH) elevation; which enhances renal 1-alpha hydroxylase activity thereby promoting conversion of available 25(OH)D to $1,25(OH)_2D$[21]. Thus, as a patient becomes vitamin D insufficient and deficient, the increase in PTH levels result in normal or elevated levels of 1, 25(OH)D. This makes the $1,25(OH)_2D$ assay useless as a measure of vitamin D status [19]. Vitamin D status is based on circulating total 25-hydroxyvitamin D [25(OH)D] concentrations, reflect both food intake and endogenous production of vitamin D.

This study has been designed with the objective to explore the association between serum 25(OH)D levels and the MetS in the middle-aged individual.

The prevalence of metabolic syndrome and associated complications like cardiovascular diseases and type2 diabetes has increased dramatically in recent years that causing a tremendous burden on medical, economic and social infrastructure. Thus, the prevention of this condition is crucial for public health. Low serum 25(OH)D levels have been linked to a range of non-skeletal health conditions in adults, including metabolic disorders and cardiovascular diseases. Therefore, lower circulating levels of serum 25(OH)D do not only inform the status of vitamin D deficiency. As far as our knowledge, few such studies have been reported to be done in our country in recent years.

OBJECTIVES

General Objectives

1. To determine serum 25(OH)D concentration in all study subjects.

Specific Objectives

The specific objectives of the study were to:

1. To measure anthropometric variables (BMI, WC) in the study subjects.
2. To measure systolic and diastolic blood pressure in the study subjects.
3. To estimate fasting plasma glucose and serum triglyceride and high-density lipoprotein cholesterol in the study subjects.
4. To compare all variables among groups based on quartiles of serum 25(OH)D levels.

METHODOLOGY

Type of study	It was a cross-sectional analytical study.
Place of study	Department of Biochemistry of Sir Salimullah Medical College, Dhaka, Bangladesh.
Study period	The study was done during the period of March'20 to July'21.
Study population	Study population included those subjects attending the outpatient department (OPD) of Endocrinology of Sir Salimullah Medical College.
Sampling technique	Purposive convenient sampling
Sample size	Total 90 subjects (45 for each group) were included for this study

Study Procedure

Subjects were selected from the outpatient department (OPD) of Endocrinology of Sir Salimullah Medical College and Mitford Hospital, Dhaka. Ethical permission was taken from the Ethical Review Committee of Sir Salimullah Medical College. After proper counselling aim, objectives, risk and procedure of the study were explained in details to all participants. Only voluntary candidates were recruited as research participants. They had the freedom to withdraw themselves from the study at any stage. Written informed consent was taken from all participants. Socio- demographic as well as other relevant data was taken and recorded in the data collection sheet with a prefixed questionnaire. A blood sample was collected for biochemical variables to be measured.

Data Collection and Processing

Before collecting specimen, each patient was interviewed and relevant information was recorded systematically in a pre-designed standard data sheet and then data was checked and edited.

Data Analysis

Continuous variables were expressed as mean values and standard deviation (SD), whereas categorical variables were described as counts and percentiles. Quartiles based on the values of 25(OH)D were created. Unpaired student t-test was used to compare the

differences in serum 25(OH)D concentrations between participants with and without MetS. Logistic regression was used to estimate the odds ratios (ORs) and 95% CIs for each quartile of serum level of 25(OH)D compared with the highest quartile value. Other statistical methods include: Analysis of variance (ANOVA) test, Chi-square test were used. The multiple linear regression analysis was used to assess the risk predictive ability of vitamin D along with other conventional risk factors of MetS. All statistical analyses were performed using SPSS version 26.0 software and $p < 0.05$ was considered as statistically significant.

RESULT

In this study, a total of 90 subjects were enrolled. Among them 45 were metabolic syndrome and 45 were without metabolic syndrome subjects.

Table I shows mean±SD of the biochemical parameters. FPG level was significantly higher in Group- I than in Group II. Serum TG and HDL-C did not differ significantly. Serum Vitamin D level was significantly lowers in Group- I than in Group II.

Table-I: Biochemical parameters of study subjects (N=90)

Variables	Group I (n=45)	Group II(n=45)	p-value
FPG (mmol/L)	7.32±2.50	5.81±1.49	0.001
TG (mg/dl)	237.78±79.86	211.29±73.20	0.105
HDLC (mg/dl)	39.80±3.87	41.16±3.10	0.070
Vitamin D (ng/ml)	26.50±5.94	30.51±5.80	0.002

Data were expressed as mean±SD.

Unpaired student t-test was performed to compare between two groups.

FPG=Fasting plasma glucose, TG=Triglyceride, HDL-C=High Density Lipoprotein Cholesterol.

Table II shows baseline characteristics of study subjects according to gender. BMI and WC were significantly higher in female than in male. Other Characteristics showed no significant difference between male and female.

Table-II: Baseline characteristics of study subjects according to gender (N=90)

Variables	Male (n=57)	Female (n=33)	p-value
Age (years)	38.88±8.55	39.33±8.28	0.806
Height (m)	1.65±0.09	1.61±0.10	0.050
Weight (kg)	68.47±14.63	70.12±8.13	0.553
BMI (kg/m2)	24.77±4.54	27.33±3.61	0.007
WC (cm)	89.23±9.88	93.88±7.22	0.020
SBP (mmHg)	110.44±14.49	111.21±12.44	0.798
DBP (mmHg)	77.19±11.92	78.48±6.67	0.569

Data were expressed as mean±SD.

Unpaired student t-test was performed to compare between male and female subjects.

Table II shows Baseline characteristics of study subjects according to quartiles of serum vitamin D. Participants were categorized into quartiles based on the values of 25(OH)D (1st quartile: <24.5, 2nd quartile: 24.5-28.12, 3rd quartile: 28.13-32.20 and 4th quartile:>32.20). Subjects in Q1 and Q2 had significantly higher weight, BMI, WC and SPB as compared to Q3 and Q4.

Table III shows biochemical parameters of study subjects according to gender. Serum Vitamin D level was significantly lowers in female than male. Other Characteristics showed no significant difference between male and female.

Table-III: Biochemical parameters of study subjects according to gender (N=90)

Variables	Male (n=57)	Female (n=33)	p-value
FPG (mmol/L)	6.28±1.66	7.06±2.82	0.099
TG (mg/dl)	221.54±72.05	229.70±86.62	0.632
HDLC (mg/dl)	40.84±2.86	39.85±4.48	0.203
Vitamin D (ng/ml)	29.94±6.25	26.01±5.26	0.003

Data were expressed as mean±SD.

Unpaired student t-test was performed to compare between male and female subjects.

Table-IV: Baseline characteristics of study subjects according to quartiles of serum vitamin D (N=90)

Variables	Quartiles of 25-(OH)D (ng/ml)				p-value
	Quartile 1 (<24.5) (n=22)	Quartile 2 (24.5-28.12) (n=22)	Quartile 3 (28.13-32.20) (n=23)	Quartile 4 (>32.20) (n=23)	
Age (years)	38.05±5.74	42.18±10.22	36.39±5.93	39.65±10.1	0.121
Height (m)	1.62±0.09	1.64±0.10	1.65±0.07	1.62±0.12	0.789
Weight (kg)	71.18±9.38	74.91±15.92	67.65±9.93	62.91±11.8	0.009
BMI (kg/m2)	27.15±3.90	28.01±5.08	24.00±3.09	23.84±3.87	0.001
WC (cm)	93.82±7.06	93.45±10.35	90.43±9.68	86.26±7.98	0.018
SBP (mmHg)	111.8±12.59	119.32±13.2	107.83±14.76	104.35±9.9	0.001
DBP (mmHg)	77.73±8.69	82.27±8.69	76.96±9.26	73.91±12.7	0.052

Data were expressed as mean±SD.

ANOVA test was performed to compare among four groups.

Table V shows biochemical parameters of study subjects according to quartiles of serum vitamin D. Subjects in Q1 and Q2 had significantly higher FPG level as compared to Q3 and Q4.

Table-V: Biochemical parameters of study subjects according to quartiles of serum vitamin D (N=90)

Variables	Quartiles of 25-(OH)D (ng/ml)				p-value
	Quartile 1 (<24.5) (n=22)	Quartile 2 (24.5-28.12) (n=22)	Quartile 3 (28.13-32.20) (n=23)	Quartile 4 (>32.20) (n=23)	
FPG (mmol/L)	8.60±2.84	6.78±1.74	5.63±0.98	5.35±1.01	0.000
TG (mg/dl)	207.45±84.2	246.82±74.4	238.61±71.58	205.5±75.1	0.167
HDLC (mg/dl)	41.14±2.36	39.73±5.30	40.13±3.29	40.91±2.59	0.518
Vitamin D (ng/ml)	20.88±3.65	26.14±1.00	30.43±1.28	36.12±3.27	0.000

Data were expressed as mean±SD.

ANOVA test was performed to compare among four groups.

DISCUSSION

This study also showed that mean 25(OH)D concentrations were lower in subjects with MetS than in subjects without MetS. This finding was consistent with the study of Miñambres *et al.*, 2012 and Ford *et al.*, 2005[22].

This study showed that those without MetS had circulating 25(OH)D levels about 30 ng/Ml. These results support the recommendation from the Endocrinology Society that optimal vitamin D status higher levels [25(OH)D] levels ≥30 ng/ml] may be protective against MetS. This finding was consistent with the study conducted by Holick *et al.*, 2011[23].

It was evident through this study that means serum vitamin D was lowered in females than males. These findings were consistent with the study of Golbahar *et al.*, 2014) and Hoteit *et al.*, 2014 [24, 25]. This could be due to the fact that women tend to spend more time indoors than men, in addition to their style of clothing and sun protection and sun avoidance attitude, which could all attribute to the vitamin D deficiency in women (Golbahar *et al.*, 2014) [24].

The percentage of low vitamin D status (<30 ng/ml) was 52% (Table VI). A study with a smaller sample (n=219) found that 60.3% had low vitamin D status (<30 ng/ml) (Caro *et al.*, 2012), while in another study (n=4,090), 68.5% had low vitamin D status (Suarez-Martinez *et al.*, 2013) [26].

Participants' belonged to lower quartiles of vitamin D had significantly higher BMI and WC, indicating that low vitamin D had an association with obesity. Similar observation was reported with the studies. Several hypotheses have been proposed to explain this association. It has been suggested that obese subjects have less exposure to sunlight, an inadequate intake of vitamin D, and decreased bioavailability of vitamin D due to enhanced uptake and clearance by adipose tissue [27, 28].

CONCLUSION

In conclusion we can say that low serum vitamin D concentration may predispose to higher BMI, WC, SBP and FPG also low serum vitamin D concentration is negatively correlated with components of MetS (WC, SBP and FPG). Low serum vitamin D concentration has significant negative relationship with BMI and FPG. Thus, lower vitamin D status can be considered as increased risk of development of metabolic syndrome.

REFERENCES

1. Holick, M. F. (2007). Vitamin D deficiency. *New England journal of medicine*, 357(3), 266–281.
2. Jalali, G., Hami, M., Namaee, N., Salehi, M., Mojahedi, M. J., & Hasanzamani, B. (2017). Relationship between vitamin D deficiency and metabolic syndrome in renal transplant patients in Mashhad, Iran. *Shiraz E Med J*, 18(3).
3. Reis, J. P., Von Mühlen, D., & Miller III, E. R. (2008). Relation of 25-hydroxyvitamin D and parathyroid hormone levels with metabolic syndrome among US adults. *European journal of endocrinology*, *159*(1), 41-48.
4. Botella-Carretero, J. I., Alvarez-Blasco, F., Villafruela, J. J., Balsa, J. A., Vázquez, C., & Escobar-Morreale, H. F. (2007). Vitamin D deficiency is associated with the metabolic syndrome in morbid obesity. *Clinical Nutrition*, *26*(5), 573-580.
5. Cheng, S., Massaro, J. M., Fox, C. S., Larson, M. G., Keyes, M. J., McCabe, E. L., ... & Wang, T. J. (2010). Adiposity, cardiometabolic risk, and vitamin D status: the Framingham Heart Study. *Diabetes*, *59*(1), 242-248.
6. Zamboni, G., Soffiati, M., Giavarina, D., & Tato, L. (1988). Mineral metabolism in obese children. *Acta Pædiatrica*, *77*(5), 741-746.
7. Yanoff, L. B., Parikh, S. J., Spitalnik, A., Denkinger, B., Sebring, N. G., Slaughter, P., ... & Yanovski, J. A. (2006). The prevalence of hypovitaminosis D and secondary hyperparathyroidism in obese Black Americans. *Clinical endocrinology*, *64*(5), 523-529.
8. Liel, Y., Ulmer, E., Shary, J., Hollis, B. W., & Bell, N. H. (1988). Low circulating vitamin D in obesity. *Calcified tissue international*, *43*(4), 199-201.
9. Danescu, L. G., Levy, S., & Levy, J. (2009). Vitamin D and diabetes mellitus. *Endocrine*, *35*(1), 11-17.
10. Salehpour, A., Hosseinpanah, F., Shidfar, F., Vafa, M., Razaghi, M., Dehghani, S., ... & Gohari, M. (2012). A 12-week double-blind randomized clinical trial of vitamin D3supplementation on body fat mass in healthy overweight and obese women. *Nutrition journal*, *11*(1), 1-8.
11. Chiu, K. C., Chu, A., Go, V. L. W., & Saad, M. F. (2004). Hypovitaminosis D is associated with insulin resistance and β cell dysfunction. *The American journal of clinical nutrition*, *79*(5), 820-825.
12. Kabadi, S. M., Lee, B. K., & Liu, L. (2012). Joint effects of obesity and vitamin D insufficiency on insulin resistance and type 2 diabetes: results from the NHANES 2001–2006. *Diabetes care*, *35*(10), 2048-2054.
13. Pinelli, N. R., Jaber, L. A., Brown, M. B., & Herman, W. H. (2010). Serum 25-hydroxy vitamin d and insulin resistance, metabolic syndrome, and glucose intolerance among Arab Americans. *Diabetes care*, *33*(6), 1373-1375.
14. Kim, J. (2015). Association between serum vitamin D, parathyroid hormone and metabolic syndrome in middle-aged and older Korean adults. *European journal of clinical nutrition*, *69*(4), 425-430.
15. Rosenblum, J. L., Castro, V. M., Moore, C. E., & Kaplan, L. M. (2012). Calcium and vitamin D supplementation is associated with decreased abdominal visceral adipose tissue in overweight and obese adults. *The American journal of clinical nutrition*, *95*(1), 101-108.
16. Fung, J. L., Hartman, T. J., Schleicher, R. L., & Goldman, M. B. (2017). Association of vitamin D intake and serum levels with fertility: results from the Lifestyle and Fertility Study. *Fertility and sterility*, *108*(2), 302-311.
17. Trummer, C., Pilz, S., Schwetz, V., Obermayer-Pietsch, B., & Lerchbaum, E. (2018). Vitamin D, PCOS and androgens in men: a systematic review. *Endocrine Connections*, *7*(3), R95-R113.
18. Szabo, A., Merke, J., Beier, E., Mall, G., & Ritz, E. (1989). 1, 25 (OH) 2 vitamin D3 inhibits parathyroid cell proliferation in experimental uremia. *Kidney international*, *35*(4), 1049-1056.
19. Holick, M. F. (2009). Vitamin D status: measurement, interpretation, and clinical application. *Annals of epidemiology*, *19*(2), 73-78.
20. Atkinson, S. A., Abrams, S. A., & Allen, L. H. (1997). Standing committee on the scientific evaluation of dietary reference intakes, food and nutrition board. *Institute of Medicine*, *1997á SearchárPubMed*.
21. Binkley, N., Ramamurthy, R., & Krueger, D. (2010). Low vitamin D status: definition, prevalence, consequences, and correction. *Endocrinology and Metabolism Clinics*, *39*(2), 287-301.
22. Ford, E. S. (2005). Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the US. *Diabetes care*, *28*(11), 2745-2749.
23. Holick, M. F. (2007). Vitamin D deficiency. *New England journal of medicine*, 357(3), 266-281. Holick, M.F. vol. 2007, pp. 366–381.
24. Golbahar, J., Al-Saffar, N., Diab, D. A., Al-Othman, S., Darwish, A., & Al-Kafaji, G. (2014). Predictors of vitamin D deficiency and insufficiency in adult Bahrainis: a cross-sectional study. *Public health nutrition*, *17*(4), 732-738.
25. Hoteit, M., Al-Shaar, L., Yazbeck, C., Sleiman, M. B., Ghalayini, T., & Fuleihan, G. E. H. (2014). Hypovitaminosis D in a sunny country: time trends, predictors, and implications for practice guidelines. *Metabolism*, *63*(7), 968-978.
26. Suárez-Martínez, E. B., Pérez, C. M., Cruz, S. K., Khorsandi, S., Chardón, C., & Ferder, L. (2013). Importance of vitamin D and vitamin D levels

status in Puerto Ricans. *Journal of health care for the poor and underserved*, *24*(4 0), 38-47.

27. Wortsman, J., Matsuoka, L. Y., Chen, T. C., Lu, Z., & Holick, M. F. (2000). Decreased bioavailability of vitamin D in obesity. *The American journal of clinical nutrition*, *72*(3), 690-693.
28. Earthman, C. P., Beckman, L. M., Masodkar, K., & Sibley, S. D. (2012). The link between obesity and low circulating 25-hydroxyvitamin D concentrations: considerations and implications. *International journal of obesity*, *36*(3), 387-396.