ESTIMATION OF SERUM VITAMIN D3 LEVEL IN METABOLIC SYNDROME PATIENTS: AN OPD BASED STUDY IN THE URBAN AREA OF BHUBANESWAR

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ABSTRACT

Background: *The metabolic syndrome is a cluster of the most dangerous heart attack risk factors: diabetes and raised fasting plasma glucose, abdominal obesity, dyslipidemia and high blood pressure. This syndrome, predominant in western part of the globe, is now encroaching in the developing countries of the eastern world very rapidly. Another emerging problem is vitamin D deficiency. Though India is a tropical country, but this problem is spreading noticeably.*
Aims & Objectives: *To estimate the level of vitamin D3 amongst the patients of metabolic syndrome and to find out any relation from the result when was compared with the level of vitamin D3 of age-sex matched control group.*
Materials & Methods: *In this cross-sectional, observational study, serum vitamin D3 levels were estimated in 150 metabolic syndrome patients of 30-60 age group as well as 150 age-sex matched normal individuals of same age group in the OPD of the Department of Medicine, Hi-Tech Medical College and Hospital, an urban area of Bhubaneswar, Odisha over a period of one and half year (September 2012 to February 2014).*
Results: *Serum vitamin D3 level is remarkably decreased in metabolic syndrome patients in comparison with that of the control group. And it is also observed that in the older age group of metabolic syndrome patients, serum vitamin D3 levels are drastically reduced in contrast to that of the same age group of controls.*
Conclusion: *From this study, we can draw a conclusion that the assessment of serum vitamin D3 in the cases of metabolic syndrome is of prime importance.*

Key words: *Metabolic syndrome, vitamin D3, Urban area.*

INTRODUCTION

The metabolic syndrome is a cluster of cardiovascular risk factors including central obesity, dyslipidemia, hypertension, insulin resistance, and glycaemic abnormalities.[1-4] It is also associated with other co-morbidities including the prothrombotic state, proinflammatory state, vascular dysregulation, and nonalcoholic fatty liver disease, hyperuricemia, polycystic ovarian syndrome (in female), erectile dysfunction (in male) and acanthosis nigricans. It is estimated that around 20-25 per cent of the world's adult population have the metabolic syndrome and they are twice as likely to die from and three times as likely to have a heart attack or stroke compared with people without the syndrome. In addition, people with metabolic syndrome have a fivefold greater risk of developing type 2 diabetes.[5] They would add to the 230 million people worldwide who already have diabetes[6], one of the most common chronic diseases worldwide and the fourth leading cause of death in the developed world.

Metabolic syndrome is an emerging global problem. Approximately one fourth of the adult European population is estimated to have metabolic syndrome, with a similar prevalence in Latin America.[7] It is also considered an emerging epidemic in developing East Asian countries, including India, China, Japan, and Korea. The prevalence of metabolic syndrome in East Asia may range from 8-13% in men and from 2-18% in women, depending on the ethnicity population and criteria used.[8-10] The number of individuals with metabolic syndrome is increasing in the countries of Asia[11], especially due to the increased consumption of westernized diet, increased stress, changed daily life style, and reduced physical activity.[12] Vitamin D, a fat-soluble vitamin, 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.[13, 14] Vitamin D has other roles in the body, including modulation of cell growth, neuromuscular and immune function, and reduction of inflammation.[13, 15, 16] Many genes encoding proteins that regulate cell proliferation, differentiation, and apoptosis are modulated in part by vitamin D.[13] Many cells have vitamin D receptors, and some convert 25(OH)D to $1,25(OH)_2D$.

AIMS & OBJECTIVES

The aim of this study was to estimate the level of vitamin D3 amongst the patients of metabolic syndrome and that result was compared with the level of vitamin D3 of control group.

MATERIAL AND METHODS

In this study, we have taken the patients of metabolic syndrome according to the definition and data from the International Diabetes Federation (IDF)[3]. According to the new IDF definition, for a person to be defined as having the metabolic syndrome they must have:

- **Central obesity:** Waist circumference was measured at the midpoint between the lower costal margin and the highest point of the iliac crest at the end of normal expiration. If BMI is >30kg/m², central obesity can be assumed and waist circumference does not need to be measured.

Plus any two of the following two factors;

- **Raised triglycerides:** ≥ 150 mg/dL (1.7 mmol/L) or specifi c treatment for this lipid abnormality.
- **Reduced HDL cholesterol:** < 40 mg/dL (1.03 mmol/L) in males, < 50 mg/dL (1.29 mmol/L) in females or specific treatment for this lipid abnormality.
- **Raised blood pressure:** Systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg or treatment of previously diagnosed hypertension.
- **Raised fasting plasma glucose (FPG):** FPG ≥ 100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes. If above 5.6 mmol/L or 100 mg/dL, Oral glucose tolerance test (OGTT) is strongly recommended but is not necessary to define presence of the syndrome.

Study Design: This was a hospital-based, observational, cross-sectional, case-control study. The study design included a prospective component as biochemical evaluations were carried out once, in a single hospital visit.

Study Area: The study was conducted at Hi-Tech Medical College and hospital, Bhubaneswar, India by the Department of Biochemistry in collaboration with the Department of Medicine.

Study Population: Patients attending outpatient department (OPD) of Department of Medicine with Metabolic Syndromes were taken as cases. After obtaining informed consent, patients attending OPD of Department of Medicine were screened for Metabolic Syndrome as per International Diabetic Federation definition. Then the cases and controls were screened for inclusion and exclusion criteria. Those found to have metabolic syndrome were recruited in the study as cases. Age & sex matched healthy controls were recruited from relatives and peers of the patients, and persons attending OPD for routine health checkups in Hi-Tech Medical College & Hospital.

Inclusion criteria for cases:

- Known metabolic syndrome patients on regular checkup.
- Known diabetic patients with central obesity and hypertension.

Exclusion criteria for cases:

- Suffering from deficiency of vitamin D or taking any sort of medicines to improve serum vitamin D level.
- Suffering from any chronic debilitating disease like malignancy, acute infection, trauma.
- Suffering from any chronic liver ailments or renal disorders.
- Pregnant women.

Criteria for controls:

Apparently healthy subjects or persons attending OPD for routine health checkup with age group of 30-60 years.

Sample size: 150 cases and 150 controls were included in this study.

Study period: September 2012 to February 2014.

Parameters for this study:

Demographic parameters- Age, Sex.

Anthropometric parameters- Waist circumference, Height, Weight, Body Mass Index (BMI).

Blood Pressure- Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP)

Biochemical Parameters- Lipid Profile (serum high density lipoprotein [HDL], low density lipoprotein [LDL], very low density lipoprotein [VLDL] and triglycerol [TG] levels) Diabetic Profile (Fasting Plasma Glucose [FPG], Post-Prandial Plasma Glucose [PPPG], and Glycosylated Haemoglobin [HbA_{1c}]) Serum vitamin D3 level.

Demographic parameters, anthropometric measurements, blood pressure and biochemical parameters were assessed in every subject of case and control groups.
A pre-designed, pre-tested, semi-structured questionnaire was used to collect various socio-demographic data like name, age, sex, address along with data about physical examination and clinical history. Heights were measured in centimeter scale using a stadiometer; fraction values were approximated to the nearest centimeter. Weights were taken in kilograms using a calibrated weighing machine and fractions were approximated to its nearest kilogram. Heights and weights were measured with individuals wearing light clothing but without shoes. Body Mass Index (BMI) or the "Quetelet Index" was calculated as per the formula of Adolphe Quetelet. According to the World Health Organization's data gathering protocol,[17] the waist circumference (in cm) was measured at the midpoint between the lower costal margin and tip of the iliac crest at the end of normal expiration, using a stretch-resistant tape that provides a constant 100 g tension. In this measurement, the tape was parallel to the floor; the individual was stood relaxed with feet close together, arms at the side and body weight evenly distributed, and little clothing to be put on. This measurement was repeated twice; if the measurements were within 1 cm of one another, the average was calculated. If the difference between the two measurements exceeds 1 cm, the two measurements were repeated.[18]

Blood pressure was assessed in the sitting position after resting for 10 min. Then it was measured again with 5-min intervals, and the average value in mmHg was used here. Blood samples were collected by venipuncture of the antecubital vein, early in the morning after an overnight fast under strict aseptic condition using dry disposable syringe & needle. From this blood samples total lipid profile, FPG, HbA_{1c} and vitamin D levels were estimated. After 2 hours of taking meal, another blood sample was taken for the estimation of PPPG same as before. Total lipid profile; FPG and PPPG were estimated by auto-analyzer ERBA EM-200. HbA_{1c} was determined by Bio-Rad D-10 Dual Program automatic analyzer. Serum vitamin D3 levels were determined by electro-chemiluminescence immunoassay using a Cobas auto-analyzer (Roche Diagnostics).

STATISTICAL METHODS

The data collected was checked for error, cleaned and double entered into MS-Excel spread sheets and checked for any entry error. Then the whole data was imported into IBM SPSS Statistics (version 20.0) and further analysis was done. Data was first summarized and then analyzed for test of significance e.g. chi-square test, independent sample student t-test wherever applicable using the software package. P value less than 0.05 was taken as significant. The whole procedures involved were transcription, preliminary data inspection, content analysis and interpretation.

RESULT

The study was conducted at Hi-Tech Medical College & Hospital, Bhubaneswar under the Department of Biochemistry in association with the Department of Medicine. One hundred and fifty patients suffering from metabolic syndrome were enrolled in the study as cases, after obtaining informed consent. Another one hundred fifty age & sex matched healthy controls were also included for comparison purpose.

Table 1: Age distribution between Case & Control group

Group	Total No.	Maximum age (Yrs.)	Minimum age (Yrs.)	Mean age (Yrs.)	Standard deviation	Standard error of mean
Case	150	58	37	46.6	5.2	0.737
Control	150	59	37	48.6	5.2	0.737

Among the cases, 66% of them were females and remaining 34% were males. Female to male ratio was 1.94:1. The minimum age recorded among the cases was 37 and the maximum was 58. The mean (±SD) age of cases was 47.7 (±5.2). Among the controls, 68% of were females and 32% were males; female to male ratio was 2.1:1. Minimum age recorded among the control group was 37 and maximum was 59. The mean (±SD) age of control group was 49.5 (±5.2).

Table 2: Age & sex distribution between Case & Control group

Parameters		Case		Control		x^2 value & d. f.	P value	Inference
		No.	(%)	No.	(%)			
Sex	Female	99	(66%)	102	(68%)	x^2= 0.162; d. f. =1	0.687	Matched
	Male	51	(34%)	48	(32%)			
Age (Yrs.)	30 to 40	36	(24%)	30	(20%)	x^2= 0.045; d. f. =2	0.832	Matched
	40 to 50	72	(48%)	60	(40%)			
	50 to 60	42	(28%)	60	(40%)			

Table 2 is showing the distribution of age and sex between case and control groups. Chi-square test shows the difference in male and female distribution between both groups were statistically insignificant (P= 0.687); similarly the difference in age distribution between groups were also insignificant (P= 0.832).

Height, weight, and waist circumference (WC) were recorded for each subject. Body mass index (BMI) was calculated from collected data. BMI was calculated by [weight in kilogram / (height in meter)^2]. Then the data was summarized by calculating the mean and SD for each parameter in both the group differently. Table 3 is showing the summarized data on anthropometric parameters for both groups.

Table 3: Summarized data on anthropometric parameters for Case and Control groups

Group	Parameters	Minimum	Maximum	Mean	SD
Case (n=150)	Height (cm)	147.3	186.7	165.9	8.8
	Weight (Kg)	63.4	103.0	82.2	9.5
	BMI (Kg/m^2)	27.8	31.7	29.8	1.1
	WC (cm)	81.7	104.2	91.2	7.2
Control (n=150)	Height (cm)	149.9	185.4	166.1	9.6
	Weight (Kg)	51.4	93.1	66.2	11.2
	BMI (Kg/m^2)	21.6	27.8	23.8	1.4
	WC (cm)	74.6	87.9	79.6	4.4

The means of the both case and control groups were compared and tested by independent sample t test for statistical significance. The mean height of the controls were slightly higher than cases, but it is not significant (P= 0.949). However, significantly higher weight (P< 0.001), BMI (P< 0.001), and waist circumference (P< 0.001) were

observed among cases in comparison to the control group. Table 4 describes the comparison of anthropometric parameters between both groups.

Table 4: Comparison of anthropometric parameters between Case and Control groups

Parameters	Mean		T statistics	P value	95% C.I. of difference	
	Case	Control			Lower	Upper
Height (cm)	165.9	166.1	-0.064	0.949	-3.77	3.53
Weight (Kg)	82.2	66.2	7.725	<0.001*	11.95	20.20
BMI (Kg/m^2)	29.8	23.8	24.02	<0.001*	5.49	6.48
WC (cm)	91.2	79.6	9.71	<0.001*	9.20	13.95

* Difference between groups is significant.

SBP and DBP were recorded for each subjects of both case and control groups. Table 5 is showing the summarized data on blood pressure for both groups.

Table 5: Summarized data on blood pressures between Case and Control groups

Group	Parameters	Maximum	Minimum	Mean	SD
Case (n=150)	SBP (mm of Hg)	120	150	135.4	6.9
	DBP (mm of Hg)	72	96	85.1	5.0
Control (n=150)	SBP (mm of Hg)	112	138	128.4	6.3
	DBP (mm of Hg)	70	90	81.8	5.6

Among the cases, 123 (82%) were on anti-hypertensive therapy, but 27 (18%) and total control group did not take any antihypertensive drugs. The means of SBP and DBP the both case and control groups were compared and tested by independent sample t test for statistical significance. Significantly higher SBP (P<0.001) and DBP (P= 0.003) were observed among cases in comparison to the control group. Table 6 describes the comparison of blood pressures between both groups.

Table 6: Comparison of blood pressures between Case and Control groups

Parameters	Mean		T statistics	P value	95% C.I. of difference	
	Case	Control			Lower	Upper
SBP (mm of Hg)	135.4	128.4	5.321	<0.001*	4.42	9.67
DBP (mm of Hg)	85.1	81.8	3.086	0.003*	1.17	5.39

* Difference between groups is significant.

The serum lipid profile was estimated for both cases and controls. The mean (±SD) of serum total cholesterol was 211.9 (±42.6) mg/dl among cases in contrast to 174.2 (±5.9) mg/dl among controls. The mean (±SD) of serum HDL-C was 39.5 (±5.6) mg/dl in the case group whereas 56.8 (±4.3) mg/dl in the control group. The mean (±SD) of serum triglyceride among the cases and controls were 229.3 (±120.3) mg/dl and 127.3 (±10.7) mg/dl respectively. Table 7 is showing the summarized data of lipid profile of case and control groups.

Table 7: Summarized data of parameters of lipid profile of Case and Control groups

Group	Parameters	Minimum	Maximum	Mean	SD
Case (n=150)	Total Cholesterol (mg/dl)	162	299	211.9	42.6
	HDL-C (mg/dl)	28	48	39.5	5.6
	LDL-C (mg/dl)	98	178	126.8	23.2
	VLDL-C (mg/dl)	22	97	45.8	24.0
	TG (mg/dl)	112	487	229.3	120.3
Control (n=150)	Total Cholesterol (mg/dl)	164	185	174.2	5.9
	HDL-C (mg/dl)	51	65	56.8	4.3
	LDL-C (mg/dl)	84	98	92.0	3.9
	VLDL-C (mg/dl)	21	29	25.5	2.1
	TG (mg/dl)	103	148	127.3	10.7

The mean (±SD) of serum LDL-C among the cases and controls were 126.8 (±23.2) mg/dl and 92.0 (±3.9) mg/dl respectively. And the mean (±SD) of serum VLDL-C was 45.8 (±24.0) mg/dl among the cases in contrast to 25.5 (±2.1) mg/dl respectively.

Among the cases, 102 (68%) were on the medication to improve the lipid profile, and rest 48 (32%) of cases and the total control group did not take any such medication.

The differences in various parameters of lipid profile were checked for statistical significance by independent sample t test. Table 8 shows the comparison of lipid profile parameters between both the groups.

Table 8: Comparison of lipid profile parameters of Case and Control groups

Parameters	Mean		T statistics	P value	95% C.I. of difference	
	Case	Control			Lower	Upper
Total Cholesterol (mg/dl)	211.9	174.2	6.2	<0.001*	25.4	49.9
HDL-C (mg/dl)	39.5	56.8	-17.3	<0.001*	-19.2	-15.3
LDL-C (mg/dl)	126.8	92.0	10.4	<0.001*	28.0	41.4
VLDL-C (mg/dl)	45.8	25.5	6.0	<0.001*	13.4	27.1
TG (mg/dl)	229.3	127.3	6.0	<0.001*	67.7	136.3

* Difference between groups is significant.

The mean of serum total cholesterol, HDL-C, LDL-C, VLDL-C, and TG – all the parameters of lipid profile are more in cases than controls and are statistically strongly significant (all p value <0.001) between cases and controls. The glycemic profile was estimated in both case and control groups. Here the glycemic profile is consists of FBS, PPBS, and HbA_{1c}. In serum FBS, the mean (±SD) were 126.0 (±11.4) mg/dl in cases and 117.0 (±7.6) mg/dl in controls. In case of serum PPBS, the mean (±SD) were 156.9 (±31.3) mg/dl and 138.4 (±7.1) mg/dl in the cases and control group respectively. The summarized data of glycemic profile of case and control groups is shown in table 9.

Table 9: Summarized data of parameters of glycemic profile of Case and Control groups

Group	Parameters	Minimum	Maximum	Mean	SD
Case (n=150)	FBS (mg/dl)	108	151	126.0	11.4
	PPBS (mg/dl)	122	287	156.9	31.3
	HbA_{1c}	6.5	8.3	7.2	0.48
Control (n=150)	FBS (mg/dl)	101	128	117.0	7.6
	PPBS (mg/dl)	123	149	138.4	7.1
	HbA_{1c}	6.0	6.4	6.2	0.14

The anti-diabetic drugs were taken by 117 (78%) of the cases, whereas the remaining 33 (22%) and total control group did not take any sort of anti-diabetic drugs. Again the differences in parameters of glycemic profile were checked for statistical significance by independent sample t test. Table 10 shows the comparison of glycemic profile parameters between both the groups.

Table 10: Comparison of parameters of glycemic profile of Case and Control groups

Parameters	Mean		T statistic	P value	95% C.I. of difference	
	Case	Control			Lower	Upper
FPG (mg/dl)	126.0	117.0	4.6	<0.001*	5.1	12.8
PPPG (mg/dl)	156.9	138.4	4.1	<0.001*	9.4	27.6
HbA_{1c}	7.2	6.2	14.1	<0.001*	0.8	1.1

* Difference between groups is significant.

Here, the mean of serum FBS & PPBS and HbA_{1c} – all the parameters of glycemic profile are more in the cases than controls and are statistically strongly significant (all p value <0.001) between cases and controls.

According to the previous data, vitamin D deficiency was defined as serum 25(OH)D levels less than 20 ng/mL;[19] vitamin D insufficiency, as ≥ 20 ng/mL to < 30 ng/mL; and vitamin D sufficiency, as ≥ 30 ng/mL.[20]

Table 11: Levels of serum vitamin D3 in case & control groups and their comparison

	Level of Serum vitamin D3 (Mean ± SD) (in ng/mL)	
Case	19.41 ± 4.94	P value
Control	27.13 ± 5.33	0.0027

From the summarized data of table 11, it was observed that the serum vitamin D3 level in case and control group were 19.41 ± 4.94 ng/mL and 27.13 ± 5.33 ng/mL respectively. They were statistically strongly significant (P = 0.0027).

After this, the case and control groups were subdivided age wise into three groups; 30-40 yrs, 40-50 yrs and 50-60 yrs. In the above age groups, cases were 63, 57 and 30 respectively, whereas controls were 65, 53 and 32 respectively. Now, age wise comparison of serum vitamin D3 level in cases and controls, the summarized data and group wise comparison were given below in table 12.

Table 12: Age group wise levels of serum vitamin D3 in case & control groups and their comparison

Level of Serum vitamin D3 (Mean ± SD) (in ng/mL)			
Age groups	Case	Control	P value
30-40 yrs	28.35 ± 2.39	33.72 ± 2.19	0.044
40-50 yrs	19.44 ± 3.12	26.45 ± 2.76	0.032
50-60 yrs	15.57 ± 3.71	23.54 ± 3.11	< 0.001

From table 12, in age group of 30-40 yrs, mean ± SD of level of serum vitamin D3 in cases and controls were 28.35 ± 2.39 ng/mL and 33.72 ± 2.19 ng/mL respectively; and these data were statistically significant (P = 0.044). In 40-50 yrs, serum vitamin D3 levels of case and control group were statistically significant (P = 0.032); as mean ± SD of level of serum vitamin D3 was 19.44 ± 3.12 ng/mL in case group and 26.45 ± 2.76 ng/mL in control group. Whereas, in the age group 50-60 yrs, mean ± SD of level of serum vitamin D3 in cases and controls were 15.57 ± 3.71 ng/mL and 23.54 ± 3.11 ng/mL respectively; and these data were statistically strongly significant (P < 0.001).

DISCUSSION AND CONCLUSION

From this study, it is observed that serum vitamin D3 levels in metabolic syndrome patients are less in comparison to that of the normal individuals; and it is statistically proven. Another point of this study is age wise distribution of serum vitamin D3 levels in metabolic syndrome patients as well as normal subjects. Here it is shown that, in all the age groups; the serum vitamin D3 levels are lesser amounts in patients' group in contrast to the normal group. Here it is clear that the level of serum vitamin D3 is decreased with the gradual progression of age. It happens in case group as well as control group. But, in comparison purpose, it is evident that vitamin D3 level is marked decreased in elder age group.

So, we must keep in mind that when we come across the patients having metabolic syndrome in OPD or in IPD, we have to estimate the serum vitamin D3 level routinely, whether it may be younger or older age group; and we should start the treatment of vitamin D deficiency simultaneously with the treatment of metabolic syndrome.

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