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A Study of Vitamin D based on BMI in medical students

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KEYWORDS

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A B S T R A C T

The purpose of this study is to investigate the changes in the levels of Vitamin D in medical students based on BMI. Vitamin D is a group of fat-soluble secosteroids responsible for intestinal absorption of calcium and phosphate. In humans, the most important related compounds of vitamin D are vitamin D2 and vitamin D3. BMI was studied in four groups namely normal BMI, Increased BMI, Obesity-I, Obesity – II. The levels of Vitamin D were found to be decreased in the Increased BMI, Obesity-I, Obesity – II medical students when compared with control Normal BMI medical students. The vitamin D-endocrine systems in obese subjects are characterized by changes consistent with secondary hyperparathyroidism and serum 1, 25-dihydroxyvitamin D.

Introduction

The body mass index (BMI), or Quetelet index, is a measure of relative weight based on an individual's mass and height. Devised between 1830 and 1850 by the Belgian polymath Adolphe Quetelet during the course of developing "social physics" (Eknoyan, Garabed 2007). It is defined as the individual's body mass divided by the square of their height – with the value universally being given in units of kg/m^2.

Vitamin D is a group of fat-soluble secosteroids responsible for intestinal absorption of calcium and phosphate. In humans, the most important related compounds of vitamin D are vitamin D2 and vitamin D3 (Holick MF, 2006).

Cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2) are unique as they constitute what we know as vitamin D and can be ingested from the diet and/or supplements (Holick MF, 2006, calvo MS *et al.*, 2005, Norman AW, 2008). The body can also synthesize vitamin D (from cholesterol) when sun exposure is adequate (hence its nickname, the "sunshine vitamin").

Vitamin D deficiency continues to be an unrecognized epidemic in many populations around the world (Holick MF, 2008). It has been reported in healthy children, young adults, middle-aged adults, and the elderly, and is common among both males and females. (Holick MF, 2006).

Materials and Methods

Chemicals

Vitamin D kits were purchased from immune Diagnostic kits, USA. All the other chemicals used were of analytical grade.

Experimental design

Out of 100 Medical students were divided in to four groups. Group I – Normal BMI (24 Students), Group-II – Increased BMI (16 Students), Group-III- Obesity-I (28 Students) and Group-IV (32 Students). The study was conducted during the period of May 2013 to July 2014 in department of clinical biochemistry, Meenakshi Medical College Hospital And Research Institute, Kanchipuram, Tamil Nadu.

Ethical concern

Ethical clearance was obtained from the Ethical committee meeting conducted at Meenakshi Medical College and Hospital on January 29th 2013.

100 randomly selected gender matched medical students and grouped according to BMI as normal with increased BMI from gender matched medical students were enrolled in the study after getting an informed consent.

The study parameters were estimated in the medical students with increased BMI and compared with the gender matched students of normal BMI.

Informed consent

An informed consent was obtained from all the subjects participating in the present study. The participants were 100 medical students studying MBBS in meenakshi medical college.The students belong to different socioeconomic and religious backgrounds.

Inclusion criteria

1. Age group of 19-21 years
2. Both sex

Exclusion criteria

1. Age <19 and > 23 years
2. Diabetes mellitus
3. Renal disorders
4. Liver pathology
5. Vitamin D supplemented individuals.
6. Any chronic illness necessitating the intake of hormones and drugs.

Anthropometry

Calculation of BMI

After removal of their footwear subjects' weight were measured with a beam balance scale, height was measured in a stadiometer to the nearest 0.5cm.their BMI was calculated using the formula

BMI = weight in kg/height in m^2

Waist circumference (cm) was taken with a tape measure as the point midway between the costal margin and iliac crest in the mid-axillary line, with the subject standing and breathing normally. Hip circumference (cm) was measured at the widest point around the greater trochanter. The waist-to-hip ratio was calculated as the waist measurement divided by the hip measurement

Biochemical parameters

Serums 25 OH vitamin D and calculate BMI levels were the biochemical parameters estimated in the study population.

Collection of blood sample

3ml of blood was collected for the estimation of biochemical parameter. The blood drawn was allowed to coagulate and the serum was separated by centrifuging and stored at -20°C until assayed.

Measurement of vitamin D

Method: 25[OH] D was measured by direct ELISA kit method

Principle

The kit used is a solid phase ELISA based on the principle of competitive binding. Anti-vitamin D antibody coated wells are incubated with vitamin D standards,controls,samples and vitamin D-biotin conjugating at room temperature for 90 mins. During the incubation, a fixed amount of biotin-labeled vitamin D competes with the endogenous vitamin D in the sample, standard, or quality control serum for a fixed number of binding sites on the anti vitamin D antibody.

Following a wash step, bound vitamin D-biotin is detected with steptavidin-HRP [SA-HRP}. SA-HRP conjugate immunologically bound to the well progressively decreases as concentration of vitamin D in the specimen increases. Unbounded SA-HRP conjugate is then removed and the wells are washed.

Next a solution of TMB reagent is added and incubated at room temperature for 30 mins, resulting in the development of blue colour. The colour development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450 nm.

A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The colour intensity is inversely proportional to the amount of 25-OH vitamin D in the sample.

The assay measures both the 25-OH vitamin D2 and vitamin D3.the total assay procedure time run time is 2.5 hours.

Materials

1. Micro well coated with anti-vitamin D
2. Vitamin D standard set: 7 vials
3. Vitamin D control set: 2 vials
4. Biotinylated 25 [OH] D reagent: 1 vial
5. Assay diluent, 1 bottle
6. steptavidin- HRP, 1 bottle
7. Stop solution, 1bottle
8. TMB substrate, 1bottle
9. Micro plate sealing film
10. Wash concentrate 20X, 1bottle

Reagents

The needed volume of standards and reagents are equilibrated to room temperature before use.51X biotin conjugate is prepared by adding 0.1ml of the 51X vitamin D-biotin conjugate to 5ml of assay dilutent. Wash buffer is prepared by adding the contents of the bottle [20X] to 475ml of distilled water.

Procedure

All reagents and specimens are brought to room temperature before use. All reagents are gently mixed without foaming. Once the procedure has started all steps are completed without interruption.

10 microlitre of 25-OH vitamin D standards, controls and samples are dispensed into each well as required. Then 200 microlitre of working solution of biotinylated 25 [OH] D reagents are dispensed into each well. The contents in the well are mixed using a plate

shaker or equivalent motion for 20 seconds. Then the shaker is covered with adhesive plate seal making sure that there is a complete seal over each well.

The sealed plates are incubated for 90 minutes at room temperature. After removing the plate seal the contents of the well are briskly shaked out in to a waste reservoir.300 microlitre of wash buffer are dispensed into each well and it is briskly shaked out in to a waste reservoir. The wells are sharply striked on absorbent paper to remove residual droplets. This procedure is repeated 2 more times for a total of 3 washes.

200 microlitre of enzyme conjugate [streptavidin-HRP] is dispensed into each well and incubated for 30 minutes at room temperature. The contents are briskly shaked out in to a waste reservoir. 300 microlitre of wash buffer is dispensed into each well and it is briskly shaked out into a waste reservoir.

The wells are striked sharply on absorbent paper to remove residuals droplets. This procedure is repeated 2 more times for a total of 3 washes. 200 microlitre of TMB substrate is dispensed in to each well and incubated for 30 mins at room temperature, preferably in the dark.50 microlitre of stop solution is dispensed into each well to stop enzymatic reaction. The plate contents are carefully mixed for 20-30 seconds. The absorbance is read on ELISA reader at 450 nm within 10 minutes of adding stop solution.

Calculation of results

The average absorbance values are calculated for each set of standards, controls and patient samples. A standard curve is constructed by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical [Y] axis and concentration on the horizontal [X] axis.

Using the mean absorbance value for each sample the corresponding concentration of the sample is determined. The values are expressed in ng/ml.

Result and Discussion

Prevalence of BMI in Medical Students

The prevalence of BMI was studied in four groups namely normal BMI (18.5 – 24.9), Increased BMI (25.0 – 29.9), Obesity-I (30.0 – 34.9), Obesity – II (35.0 – 39.9). Out of 100 Medical students 16 had increased BMI, 28 belonged to Obesity –I group and 32 belonged to obesity-II group while 24 had normal BMI. The percentage prevalence of BMI in normal, Increased BMI, Obesity-I and Obesity II levels medical students is shown as a pie chart in Chart 1.

The percentage of normal, Increased BMI, Obesity-I and Obesity II levels in the groups are 24%, 16%, 28% and 32% respectively and BMI levels are 21.38, 27.52, 33.21 and 38.54respectively. In our study, BMI was estimated in medical students of 19-21 years without any history of signs and symptoms of clinical diseases, studying in Meenakshi Medical College, Enathur, Kanchipuram.

Characteristics of the study population

According to BMI levels, the characteristics of the entire study population such as normal BMI, Increased BMI, Obesity-I and Obesity II levels were listed in Table 1. Among the total study participants, Normal BMI levels were seen in 24 % increased BMI in 16%, obesity-I in 28% and Obesity-II in 32%. The sufficient Vitamin D levels

were seen among 95.83% of students with ideal BMI. In addition to 4.16% of students with increased BMI, 0% and 0% in obese I and II respectively. The duration of exposure to sunlight, less than 15 min and more than 15 min in medical students with sufficient Vitamin D level were approximately 21.05% and 95.83%. And also the time of exposure in students before 11am, 11am – 3pm and after 3pm with sufficient Vitamin D level was approximately 25%, 54.1% and 20.8% respectively.

Among the participants with sufficient Vitamin D level, 20.8% of them were not shown any physical activity, 50% of them shown occasional and 29.1% of them shown regular physical activity.

As shown in table 1 low Vitamin D level were seen nearly among 19.7% of increased BMI students, 36.84% of Obesity-I and 42.1% of Obesity-II respectively.

The duration of exposure to sunlight less than 15 min and more 15 min and time of exposurebefore11am, 11 am – 3 pm and after 3 pm in students with Hypovitaminosis D were 78.94%, 4.1%, 11.84%, 53.94% and 35.5% respectively. No physical activity was observed among 36.54% of students with low Vitamin D levels.

The observed results in Table 5 clearly reveals that 16% (n=16) of the participants were found to have Vitamin D level between 20-30ng/ml (Increased BMI) and 28% (n=28) of the participants were found to have Vitamin D level <25 ng/ml (Obese-I) and 32% (n=32) of the participants have Vitamin D level <20ng/ml (obese-II) and also 24% (n=24) of the study participants have Vitamin D levels between 30-40ng/ml The observed mean Vitamin D status of Normal BMI, Increased BMI, Obese-I and Obese-II students were 37.02±4.40, 30.75±3.10, 24.12±2.50 and 18.06±1.90 respectively.

Vitamin D deficiency continues to be an unrecognized epidemic in many populations around the world (Holick MF, Chen TC, 2008). It has been reported in healthy children, young adults, middle-aged adults, and the elderly, and is common among both males and females (Holick MF, 2006).

Prevalence of BMI In Medical Students

The body mass index (BMI] or Quetelet index, is a measure of relative weight based on an individual's mass and height. It is defined as the individual's body mass divided by the square of their height – with the value universally being given in units of kg/m^2.

In our study we observed that out of 100 Medical students 16 had increased BMI, 28 belonged to Obesity –I group and 32 belonged to obesity-II group while 24 had normal BMI. The percentage of normal, Increased BMI, Obesity-I and Obesity II levels in the groups are 24%, 16%, 28% and 32% respectively and BMI levels are 21.38, 27.52, 33.21 and 38.54respectively.

The prevalence of overweight and obesity among the medical students in the present study was similar to that of the general population in India. In our study, 71.7% of undergraduate medical students were obese which was almost similar to study conducted in West Bengal in India among undergraduate medical students (Park K. Text book of preventive and social Medicine 19th edition page 335).The international diabetes foundation has accepted BMI > 25kg/m2 and >23kg/m2 as cut off value for obesity for Asian men & womenrespectively7 and according to this

the prevalence of Obesity among males was 32%and among females was 52% which was alarming.

Study of vitamin D based on BMI

In our study sufficient vitamin D levels were seen among 95.83% of students with ideal BMI. 19.7% of the study participants had hypovitaminosis with increased BMI and 36.84% of study population had hypovitaminosis in the obesity I group whereas 42.1 % had hypovitaminosis in the obesity II group. From the results obtained in our study it is clear that hypovitaminosis is seen among students with increased BMI and in obese students which was similar with the previous studies conducted by (Parikh *et al*., 2004) and by (konradsen *et al*., 2008).

Vitamin D has been suggested to be a potential factor in the prevention of many illnesses, including some cancers, autoimmune disorders, hypertension, diabetes and, even more speculatively, perhaps obesity itself(Worstman J *et al.*, 2000).

There are several potential mechanisms by which obesity could contribute to decreased serum 25-hydroxyvitamin D levels. Some investigators have suggested that sequestration of vitamin D by adipose tissue contributes to low circulating25-hydroxyvitamin D concentrations in obese individuals (konradsen *et al*., 2008, Worstman J *et al.*, 2000). There appears to be increased uptake and storage of vitamin D, which is fat-soluble, by the adipose tissue of obese individuals relative to that in lean individuals (konradsen *et al*., 2008).

Blum et al. subsequently measured adipose tissue vitamin D3 concentrations in 17 obese subjects by liquid chromatography mass spectrometry and reported a strong inverse relationship between amount of fat tissue and serum vitamin D3 concentration, providing further evidence of fat tissue vitamin D storage in obese individuals.

Li J et al. has suggested additional potential reasons for obesity as a contributor to decreased circulating 25-hydroxyvitamin D. One hypothesis is that there may be increased catabolism of vitamin D with increasing adiposity owing to the local action of the 24-hydroxylase enzyme that has been found in human adipose tissue (Li J *et al.,* 2008). Another hypothesis is that synthesis of 25-hydroxyvitamin D by the liver may occur at a lower rate in obese individuals relative to that in lean individuals.

A reported study shows that the prevalence of VDD is approaching 90% due to their limited exposure to sunlight which supports our study(JawadMunir,MD *et al.*,2001).In addition, from our study, lack of physical activity was observed among almost 37% of the total hypovitaminosis D population, will also contribute to high prevalence of low vitamin D levels. Thus from our study, it is clearly understood that decreased exposure to sunlight and less outdoor physical activity can have great effects on vitamin D levels.

Statistical analysis

For statistical analysis, one way analysis of analysis of Variance (ANOVA) was used, followed by the Newman-Keuls Multiple Comparison test.

From the present study, we concluded that the normal BMI, regular physical activity and more than 15mins sun exposure were significantly increased Vitamin D level group – I normal BMI medical students when compared with other groups like increased BMI, Obesity I and Obesity II medical students.

Table.1 Characteristics of the Study Population

	Overall	%	Sufficient Vitamin D N=24	%	Hypovitaminosis D N=76	%
BMI						
Ideal	24	24	23	95.83		
Increased BMI	16	16	1	4.16	15	19.7
Obese I	28	28	0	0	28	36.84
Obese II	32	32	0	0	32	42.1

Table.2 Characteristics of study population

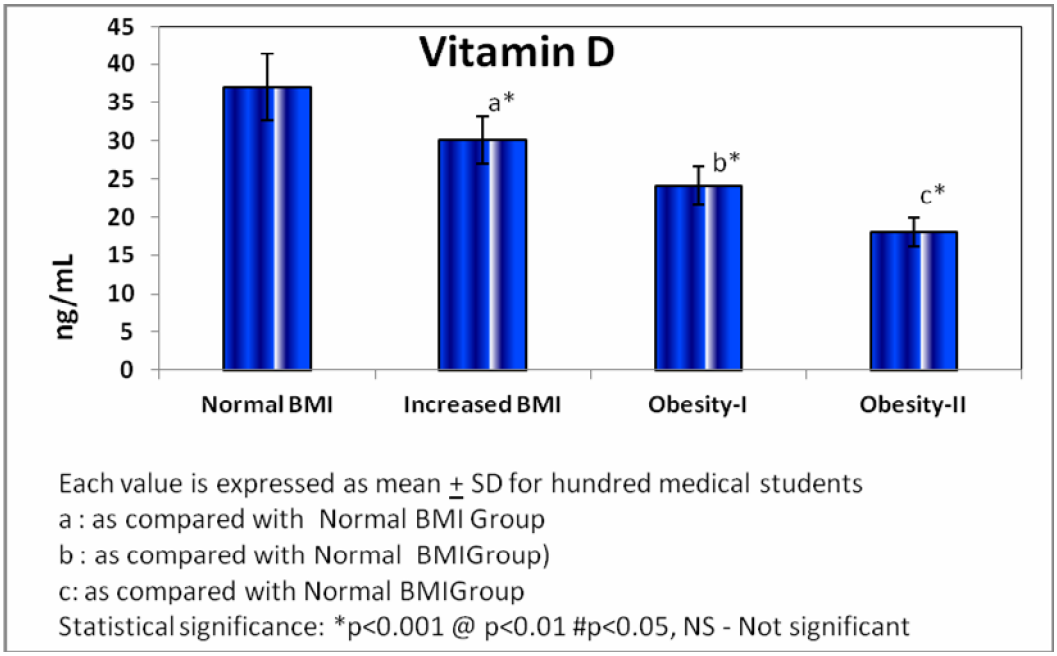
	Overall	%	Sufficient Vitamin DN=24	%	Hypovitaminosis D N=76	%
Sun exposure						
<15 min	76	76	16	21.05	60	78.94
>15 min	24	24	23	95.83	1	4.1
Time of exposure						
Before 11 am	14	14	6	25	9	11.84
11 am - 3 pm	54	54	13	54.1	41	53.94
After 3 pm	32	32	5	20.8	27	35.5
Physical activity						
Nil	33	33	5	20.8	28	36.84
Occasional	47	47	12	50	35	46.05
Regular	20	20	7	29.1	13	17.10

% - percentage of study population

Table.2 Study of Vitamin D based on BMI

Particulars	Normal BMI	Increased BMI	Obese-I	Obese-II	P valve
Number of participants	24	16	28	32	NA
Percentage of study population	24%	16%	28%	32%	NA
25(OH)D	37.02±4.40	30.75±3.10	24.12±2.50	18.06±1.90	0.000*[a,b]

a-Comparison between normal BMI and increased BMI
b-Comparison between normal BMI and Obese-I
c- Comparison between Normal BMI and Obese-II
P< 0.001 is considered significant
NS- Not significant
NA- Not applicable



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