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Research Article

A CROSS SECTIONAL STUDY TO DEFINE THE STATE OF VITAMIN D₃ IN HEALTHY PERSONS

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Abstract:

Objectives: The aim of our study was to Define the deficiency of vitamin D₃ and inefficiency in clinically asymptomatic and seemingly in healthy young persons.

Study Design: A cross sectional study.

Study and Time Duration: This study was conducted in Department of Medicine, Benazir Bhutto hospital (BBH) Rawalpindi for the duration of one year starting from July, 2019 to June, 2020.

Methodology: According to our study criteria we selected the sample of 100 volunteer participants in which male sample was 57 and female sample was 43. We collected 5ml venous blood in a disposable syringe. We use standard laboratory procedures to estimate phosphate, serum calcium, serum creatinine and blood urea. Vitamin D₃ is detected by ARCHITECT I 1000 system. SPSS 20 was used for analysis of data.

Results: Serum Calcium and Phosphate were noted as 3.23 ± 0.85 and 3.25 ± 0.80 mg/dl with P-value equal to 0.710 and 8.76 ± 1.11 , 9.20 ± 0.2 mg/dl with P-value equal to 0.036 in male and female subjects respectively. Vitamin D₃ (mean \pm SD) in male and female was noted as 23.0 ± 8.24 and 27.19 ± 14.13 ng/dl (P=0.006). Vitamin D₃ (mean \pm SD) of total study population was noted as 24.80 ± 11.29 ng/ml. Frequency of vitamin D₃ deficiency, insufficiency and sufficiency noted in 45%, 32% and 23% respectively (P=0.0001).

Conclusion: The present study showed that vitamin D₃ deficiency and insufficiency in 77% of young apparently healthy and clinically asymptomatic adults. Normal vitamin D₃ levels were noted in only 23% of subjects. Furthermore, studies are recommended and vitamin D₃ supplements should be described by clinicians.

Key words: Iron, TIBC, Ferritin, Helicobacter Pylori, vitamin D₃.

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INTRODUCTION:

Vitamin D₃ (cholecalciferol) is a sunshine fat-soluble vitamin of ketosteroid family. Vitamin D₃ is activated by liver and kidneys into 1,25- dihydroxy cholecalciferol that functions as a hormone and is essential for the bone health [1]. Currently, Vitamin D₃ deficiency has been reported from World over. But its deficiency is highly overlooked and ignored health problem. Vitamin D₃ deficiency has become an epidemic health problem. Approximately, one billion people are suffering from Vitamin D₃ deficiency. Vitamin D₃ deficiency is prevalent in Pakistan [2]. A previous study reported 53.5% frequency of Vitamin D₃ deficiency in the populations [3]. Vitamin D₃ deficiency is prevalent in all age groups from the neonates to the grown-up elderly populace. Pregnant women are at increased risk of vitamin is naturally synthesized in skin by ultraviolet rays of sun light beside its dietary supply.

Social and financial factors are major contributing factors in Vitamin D₃ deficiency in Pakistan. Malnutrition is a major public problem of developing countries where there is want of a balanced diet in the community. Financial factor is a major barrier in obtaining sufficient micro-nutrients in diet [4]. Social factors also contribute to Vitamin D₃ deficiency such as the confinement of women to the home and wearing of traditional clothes are additional aggravating factors in developing countries in particular the Pakistan [5]. Vitamin D₃ is reported to regulate more than 200 genes in its active form [6]. The 1,25- dihydroxy cholecalciferol is essential for the bone remodeling, mineralization, calcium absorption form gut, and regulation of phosphate.

Plasma levels of 25- hydroxy cholecalciferol range 30 - 50 ng/ml. Serum levels < 20 ng/ml are considered as vitamin D₃ deficiency. Clinically, vitamin D₃ deficiency remains asymptomatic till its plasma levels too low to create clinical manifestations. Clinically, Vitamin D₃ deficiency presents as bone pain, fractures, muscle weakness, calcium and phosphate disturbed levels, and hyperparathyroidism. Long standing Vitamin D₃ deficiency causes severe bone disease called the rickets in childhood and osteomalacia [7]. Recently, Vitamin D₃ deficiency has been linked to the diabetes mellitus, migraine, cancer, and coronary artery disease, etc. [8,9]. Vitamin D₃ deficiency is most under diagnosed and under estimated health problem. Vitamin D₃ deficiency is very common in Pakistan, but majority of people don't care because of its asymptomatic course [10]. The present study was planned to determine serum vitamin D₃ levels of deficiency, sufficiency and insufficiency in clinically

asymptomatic and apparently healthy young adult male.

METHODOLOGY:

This cross-sectional study was conducted in Department of Medicine, , Benazir Bhutto hospital Rawalpindi for the duration of one year starting from July, 2019 to June, 2020.

Ethical permission was taken from the institution's review committee in writing. Participants were selected from the attendants coming with the patients in outpatient and inpatient departments. A sample of 100 volunteer participants, comprising of 57 male and female was selected according to the study criteria. Young adults of 20- 40 years of age and both male and female without any history of medical and surgical problems were included. Adults with acute or chronic systemic disease were excluded by proper history and examination. Participants with concomitant systemic disease such as diabetes mellitus, malabsorption syndrome, inflammatory bowel disease, chronic diarrhea, etc. were excluded. Participants with history of multivitamin and multivitamin supplements intake were excluded too.

Finally, a sample 57 male and 43 females fulfilling the study criteria who gave written consent were selected for the study protocol. Sampling technique for participant's selection used was non- probability (purposive) sampling. Study obeyed the criteria of inclusion and exclusion strictly. Participants were interviewed with full confidence. They were informed that the study will cause no damage to them neither physical nor financial loss. Participants were given 3 days to consider for the enrollment into the study protocol. They were given option of leaving the study protocol at any time without telling the reason. For laboratory investigations, they were informed to ask any question. Participants were informed they don't have to pay for the laboratory investigations. Educated volunteer subjects who fulfilled the criteria qualified for the research protocol. Participants were informed that study will include volunteer subjects who sign a consent form unconditionally.

Volunteers were given consent form to sign. Biodata and laboratory results were noted in a predesigned pre structured proforma. Confidentiality was maintained by keeping participants data in lockers. Continuous data was presented as Mean±SD by application of Student's t-test. And categorical variables were presented as frequency and percentage by application of Chi square test. SPSS 20 was used for analysis of

data. Statistical significance was defined as $P \leq 0.05$ (95% Confidence interval).

Volunteers were asked for their cooperation and to comply with the researcher. Volunteers were examined by a medical officer and a consultant physician. Volunteers were asked for blood sampling on the day they were ready. They were ensured that the blood will be used for the laboratory investigation purpose only. Volunteers were asked to expose the ante cubital fossa. Prominent vein was located and marked. Skin was sterilized with alcohol gauze. 5ml venous blood was collected in disposable syringe. Centrifugation was performed at 3000 rpm (10

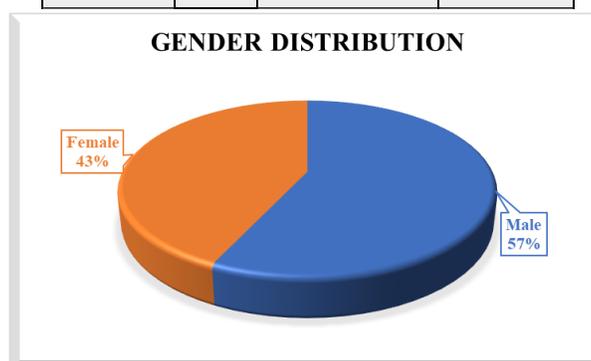
minutes. Sera were separated out for the biochemical analysis. Sera were stored at -20-degree Celsius if analysis was delayed. Blood urea, serum creatinine, serum calcium and phosphate were estimated by standard laboratory procedures. ARCHITECT I 1000 system detected the vitamin D₃.

RESULTS:

Out of 100 participants, 57% were male and 43% were female ($P=0.067$) (Table-I). Age (mean \pm SD) of male and female was noted 31.79 \pm 10.55 and 29.00 \pm 9.24 years respectively ($P=0.17$). Study subjects were body weight and blood pressure matched as shown in Table-II ($P>0.05$).

Table No 01: Gender Distribution

Gender	No.	Percentage	P-value
Male	57	57%	0.067
Female	43	43%	
Total	100	100	



Renal function was found normal as shown by blood urea and serum creatinine (table 1, $P>0.05$). Serum Calcium and Phosphate were noted as 8.76 \pm 1.11 and 9.20 \pm 0.2 mg/dl ($P=0.036$) & 3.23 \pm 0.85 and 3.25 \pm 0.80 mg/dl ($P=0.71$) in male and female subjects respectively.

Table No 02: Physical and Laboratory Findings of Study Subjects

Statistics	Male	Female	P-Value
Age (years)	31.79 \pm 10.55	29.00 \pm 9.24	0.17
Body weight (Kg)	74.73 \pm 10.50	76.28 \pm 11.71	0.49
Systolic BP (mmHg)	130.26 \pm 9.21	128.35 \pm 9.01	0.96
Diastolic BP (mmHg)	68.58 \pm 4.77	69.56 \pm 7.27	0.42
Urea (mg/dl)	25.33 \pm 7.18	23.44 \pm 6.40	0.57
Creatinine(mg/dl)	0.98 \pm 0.22	0.91 \pm 0.23	0.78
Calcium (mg/dl)	8.76 \pm 1.11	9.20 \pm 0.2	0.036
Phosphate(mg/dl)	3.23 \pm 0.85	3.25 \pm 0.80	0.71
VitaminD ₃ (ng/dl)	23.0 \pm 8.24	27.19 \pm 14.13	0.006

Vitamin D₃ (mean \pm SD) in male and female was noted as 23.0 \pm 8.24 and 27.19 \pm 14.13 ng/dl ($P=0.006$). Vitamin D₃ (mean \pm SD) of total study population was noted as 24.80 \pm 11.29 ng/ml. Vitamin D₃ deficiency (<20 ng/mL),

insufficiency (20- 30 ng/ mL) and sufficiency (>30 ng/mL) were noted as 16.17 ± 2.43 , 23.34 ± 1.47 and 43.69 ± 5.50 ng/mL respectively ($P=0.0001$) (Table-III).

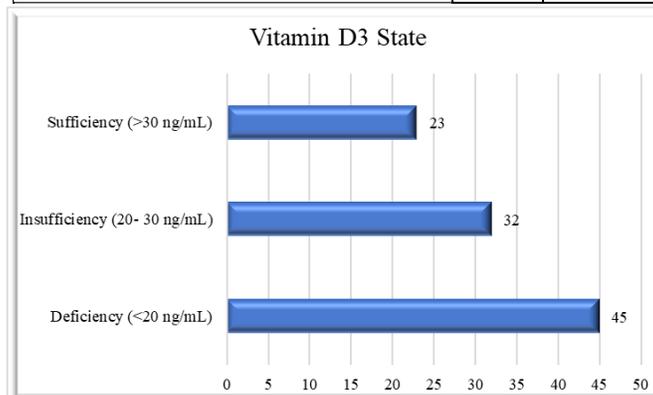
Table No 03: Vitamin D₃ Distribution

Vitamin D ₃	Mean± SD
Deficiency (<20 ng/mL)	16.17±2.43
Insufficiency (20- 30 ng/mL)	23.34±1.47
Sufficiency (>30 ng/mL)	43.69±5.50
Total	24.80±11.29

Table 4 shows the frequency of vitamin D₃ deficiency, insufficiency and sufficiency noted in 45%, 32% and 23% respectively ($P=0.0001$) (Table-IV).

Table No 04: Frequency of deficiency, insufficiency and sufficiency in vitamin D₃

Category	Qty	%age
Deficiency (<20 ng/mL)	45	45%
Insufficiency (20- 30 ng/mL)	32	32%
Sufficiency (>30 ng/mL)	23	23%
Total	100	100%



DISCUSSION:

The purpose of this study was to reporting on the vitamin D₃ deficiency and insufficiency in apparently healthy young adult subjects. In present study, vitamin D₃ deficiency and insufficiency were noted in clinically asymptomatic and apparently healthy young adults. The study included both male and female subjects. Of 100 participants, 57% were male and 43% were female ($P=0.067$) (Table 1). The study subjects were body weight and blood pressure matched with normal renal function as detected by urea and serum creatinine. Vitamin D₃ is a major public health problem of many developing countries [11,12]. Vitamin D₃, like other health problems is a hidden problem which remains unnoticed till the subjects develop clinical symptoms. Now Vitamin D₃ is a challenging public health issue of developing countries including Pakistan. Vitamin D₃ deficiency as

a challenging public health problem was notified by the National Nutrition Survey (2011) for the first time [13].

The present study selected young apparently healthy male and female subjects for vitamin D₃ estimation. Mean± SD age of male and female was 31.79 ± 10.55 and 29.00 ± 9.24 years respectively ($P=0.17$). These findings are discordant to previous studies because young adult subjects were selected by inclusion criteria in the present study [14,15]. Serum Calcium and Phosphate were noted as 8.76 ± 1.11 and 9.20 ± 0.2 mg/dl ($P=0.036$) & 3.23 ± 0.85 and 3.25 ± 0.80 mg/dl ($P=0.71$) in male and female subjects respectively. These findings are in concordant with previous studies [16,17].

We found mean (SD) Vitamin D₃ in male and female as 23.0 ± 8.24 and 27.19 ± 14.13 ng/dl ($P=0.006$) and

vitamin D₃ in total study subjects as 24.80±11.29 ng/ml. In present study, the frequency of vitamin D₃ deficiency was 45%, insufficiency 32% and sufficiency was noted in only 23% subjects (P=0.0001). The findings suggest prevailing vitamin D₃ deficiency and results are consistent with previous studies reported from Pakistan [18,19]. A recent study had reported total 63.4% vitamin D₃ deficiency and 14.9% insufficiency. Only 21.8% subjects had normal vitamin D₃ levels. In present study vitamin D₃ sufficiency was noted in only 23% subject that is in full agreement with 21.8% deficiency reported by above study. The findings of present study are also supported by previous study that reported vitamin D₃ deficiency, insufficiency and sufficiency in 53.5%, 31.2% and 15.3% of respectively.

A previous study had reported vitamin D₃ deficiency in 98.86% of their study subjects that is very high prevalence. Although results are inconsistent with present study, but it is believable that such high population pockets of vitamin D₃ deficiency are prevalent in the country due to prevailing malnutrition. Another reason of low frequency of vitamin D₃ deficiency of present study is most probably the young apparently health subjects were selected and studied for vitamin D. Evidence based findings reveal hypovitaminosis D is prevalent in the country. High frequency of vitamin D₃ deficiency is consistent with other previous studies [20,21]. In present study, the vitamin D₃ deficiency was noted in both male and female noted as 23.0±8.24 and 27.19±14.13 ng/dl.

Vitamin D₃ deficiency was found statistically significant between male and female subjects (P=0.006). This finding is inconsistent with previous studies reported from Pakistan. Jadoon et al [18] reported statistically non-significant difference for the male and female gender that is inconsistent to our findings. Keeping this scenario in view, it is concluded that the vitamin D₃ deficiency is prevalent in normal health adults but remains unnoticed due to poor health seeking behavior of society. We think only limitation of present study is a small sample size. The strengths of study include the; prospective study design, inclusion and exclusion criteria, normal renal functions and evidence-based findings supported by previous studies from the country. However, our findings cannot be generalized to other geographical areas with better economic status and health screening facilities.

CONCLUSION:

The current study showed that vitamin D₃ deficiency and insufficiency was there in 77% of young

apparently healthy and clinically asymptomatic persons. Normal vitamin D₃ levels were noted in only 23% of subjects. Furthermore, studies are recommended and vitamin D₃ supplements should be described by clinicians.

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