INTRODUCTION

Vitamin D₃ (cholecalciferol) is a sunshine fat soluble vitamin of secosteroid 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.¹² 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 D₃ deficiency due to the malnutrition.⁴ Vitamin D₃ 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.⁵ 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.⁶ 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.

ABSTRACT… Objectives: Determination of vitamin D₃ deficiency and insufficiency in clinically asymptomatic and apparently healthy young adult male. Study Design: Cross sectional study. Study setting & Duration: Department of Medicine, Layari General Hospital Shaheed Muhtarma Benazir Bhutto Medical College from March 2015 to May 2016. Subjects and Methods: A sample of 100 volunteer participants, comprising of 57 male and female was selected according to the study criteria. 5 ml venous blood was collected in disposable syringe. Blood urea, serum creatinine, serum calcium and phosphate were estimated by standard laboratory procedures. ARCHITECT I 1000 system detected the vitamin D₃. Data analysis was analysed by SPSS 22.0 (USA). Results: 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=0.036) & 3.23±0.85 and 3.25±0.80 mg/dl (P=0.036) in male and female subjects respectively. Vitamin D₃ (mean± 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: We found vitamin D₃ deficiency and insufficiency in 77% of young apparently healthy and clinically asymptomatic adults.

Key words: Helicobacter Pylori, Ferritin, Iron, TIBC, Male.
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. Recently, Vitamin D₃ deficiency has been linked to the diabetes mellitus, migraine, cancer, and coronary artery disease, etc. 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. 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.

SUBJECTS AND METHODS
The present cross sectional study was designed and conducted to analyze the vitamin D₃ levels in clinically asymptomatic healthy young adult male. The study was conducted at the Department of Medicine, Layari General Hospital Shaheed Muhtrama Benazir Bhutto Medical College from March 2015 to May 2016. Prior 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 diarrhoea, etc were excluded. Participants with history of multivitamin and multivitamin supplements intake were excluded too. Finally a sample 57 male and 47 female 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 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. 5 ml 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°C 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. Volunteers were given consent form to sign. Biodata and laboratory results were noted in a pre- designed pre structured proforma. Confidentiality was maintained by keeping participants data in lockers.

Data analysis was analysed by SPSS 22.0 (USA).
Continuous data was presented as mean ± SD by application of Student’s t-test. And categorical variables were presented as frequency and % by application of Chi–square test. Statistical significance was defined as P ≤ 0.05 (95% Confidence interval).

RESULTS
Age (mean ± SD) of male and female was noted 31.79±10.55 and 29.00±9.24 years respectively (P = 0.17). Study subjects were body weight and blood pressure matched as shown in Table-I (P > 0.05). 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±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. Of 100 participants, 57% were male and 43% were female (P=0.067) (Table II). Vitamin D₃ (mean± 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± SD) of total study population was noted as 24.80± 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 4 shows the frequency of vitamin D₃ deficiency, insufficiency and sufficiency noted in 45%, 32% and 23% respectively (P=0.0001) (Table IV).

DISCUSSION
The present is the first study 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 2). 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. 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. 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 studies¹³,¹⁴ because young adult subjects were selected by inclusion criteria in the present study. 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.¹⁵-¹⁷

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.²,¹⁸ Jadoon et al¹⁸ 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 economical status and health screening facilities.

CONCLUSION
The present study reports 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. Further studies are recommended and vitamin D₃ supplements should be prescribed clinicians.

REFERENCES


**AUTHORSHIP AND CONTRIBUTION DECLARATION**

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