Original Article

Serum cytokines and bone turn over markers: A cross sectional study in Karachi Pakistan

Arfa Azhar[1], Sheraz Hakeem[1], Kamran Afzal[1], Sumaira Riffat[2], Mussarat Ashraf[1], Rehana Rehman[1]

[1]Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan.
[2] Jinnah Sindh Medical University, Karachi, Pakistan.

ABSTRACT

Objectives: To predict marker of osteoporosis and osteopenia in female population of Karachi, Pakistan.
Methodology: This cross-sectional study was performed from June 2017 to August 2019 in "Department of Biological and Biomedical Sciences, Aga Khan University Hospital" on 245 female subjects, age range 25 to 70 years; categorized into normal (T-score ≥ -1), osteopenic (T-score -1.1 to -2.4) and osteoporotic (T-score <-2.5) by dual-energy X-ray absorptiometry (DEXA) scan.Vitamin D (VD), Vitamin D Binding Protein (VDBP), Tumor Necrosis Factor Alpha (TNF α), Interleuking-6 (IL-6), Osteocalcin and C-Terminal Telopeptide (CTX) were analyzed by Enzyme Linked Immunosorbent Assay. Analysis of Variance (ANOVA) and spearman's rank correlation test was performed to compare the difference and association among groups. Regression analysis used to predict factors responsible for osteopenia /osteoporosis.
Results: There were 105 (42.9%), 84 (34.3%) and 56 (22.8%) females in normal, osteopenia and osteoporosis groups respectively. The mean serum values of VD, TNF, IL-6 and CTX were found to be significantly diverse in all three groups ($p<0.005$). VD had a significant negative correlation with BMI, TNF, IL-6, osteocalcin and CTX. A robust positive correlation was observed between VD and VDBP ($r= 0.596$, $p<0.001$).
It was noted that with every one unit increase in BMI the prevalence of osteopenia was decreased by 72% ($p<0.05$) and prevalence of osteoporosis was significantly decreased by 66.2% ($p<0.05$) and with every one unit increase in VDBP levels the prevalence of osteoporosis was significantly decreased by 56.4% ($p<0.05$).
Conclusion: BMI and VDBP levels were found to be predictive factors for osteopenia and osteoporosis. These findings emphasize on the importance of monitoring Vitamin D levels, BMI, and VDBP to assess bone health and predict the risk of osteoporosis and osteopenia in the female population of Karachi, Pakistan.

KEY WORDS: Vitamin D (VD), Vitamin D Binding Protein (VDBP), Tumor Necrosis Factor Alpha (TNF α), Interleuking-6 (IL-6), Osteocalcin and C-Terminal Telopeptide (CTX), Body mass index (BMI).

INTRODUCTION

Osteoporosis and osteopenia can be described as a progressive loss of bone mass with the increasing age of an individual. Osteoporosis has been characterized by microarchitectural destruction of bone structure and decreased bone strength, On the other hand, osteopenia can be described as decreased bone density up to a stage that does not increase fracture risk in an individual.[1]

The impaired microarchitecture of bone leads to reduced bone mineral density and bone strength. Osteoporosis is characterized by bone mineral density (BMD) measurements falling below one standard

Address for Correspondence: Dr. Rehana Rehman, Ph. D. Physiology, FHEA (UK), MHPE, Bahadur Ali Kamruddin Jessani Endowed Professor, Professor in the Department of Biological & Biomedical Sciences, Director of MPhil, Chair Faculty , Appointment Committee-BBS, Chair Faculty Mentorship Forum, Aga Khan University, Karachi - Pakistan.

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deviation from the mean but greater than 2.5 standard deviations below the mean (T-score <-1 and >-2.5 SD) for young, healthy adults.[2]

Across the world, Asia is known to have reported the lowest t-scores. Although osteopenia and osteoporosis are known to affect individuals of all age groups and ethnic backgrounds, it is more common in non-Hispanic white males and females and Asian women particularly high in postmenopausal women.[3] According to studies about the global prevalence of osteoporosis, Iran has shown the highest prevalence with 77.3% and Canadian studies have shown the least with 10.7%.[4] A large majority of postmenopausal women in Pakistan suffer from osteoporosis which is known to increase with increasing age.[5] A recent study in the Khyber Pakhtunkhwa (KPK) province of Pakistan reported that 47.7% of women had osteopenia and 24.7% suffered from osteoporosis.[6] Similar prevalence rates were observed in a study in Karachi.[6] Hence it can be concluded that a decrease in BMD and its related disorders are a major health concern.

Insufficient serum vitamin D (VD) is related to increased bone turnover and bone loss. In the elderly, a more vulnerable population, many predisposing factors contribute to insufficient VD level.[7] Vitamin D binding protein (VDBP) is responsible for maintaining serum VD levels because it acts as a VD transporter molecule to target tissues and therefore regulates the availability of unbound VD. Bone turnover is procedure of bone resorption pursued by formation of sole bone. Osteoblasts participate in formation of new bone, whereas osteoclasts cause bone resorption. The enhanced ability of bone resorption by osteoclasts contributes significantly to the development of osteopenia and osteoporosis. Research has shown that certain pro-inflammatory cytokines particularly Tumor Necrosis Factor Alpha (TNF α), Interleukin-6 (IL-6), Osteocalcin and C-Terminal Telopeptide (CTX), mediate the bone remodeling process and contribute to the etiology of osteoporosis later in life. The bone quality is affected by bone turnover.[6] Bone turnover or bone remodeling is the balance between old bone regeneration and new bone formation. When bone is damaged, osteoclasts contribute to bone regeneration and form remodeling cavities on the cortical and endosteal surfaces of bones, while osteoblasts are responsible for filling resorption cavities. This is followed by a rapid primary mineralization and then a slow secondary mineralization which adds on to the mineral content of the bone matrix.[8]

Clinical, medical, behavioral, nutritional, and genetic variables all contribute to osteoporosis. As people age the bone mass decreases and due to the fast-growing elderly population, the economic and social burden of osteoporosis is rising.[9] There is lack of sufficient data to support the role of these factors in decreased BMD which subsequently means there is lack of awareness amongst not just health care practitioners but also the general public.

The objective of our study is to predict markers of osteoporosis and osteopenia in female participants presenting at a tertiary care hospital of Karachi, Pakistan.

METHODOLOGY

This cross-sectional study was performed from June 2017 to August 2019 in "Department of Biological and Biomedical Sciences" with approval of the "Aga Khan University Ethics Review Committee 4146- BBS-ERC-19". The sample size (245) was calculated by the "Open Epi Sample Size Calculator", 99% confidence interval and 5% margin of error. We selected females with age range 25 to 70 years from OPD of Aga Khan University.

Healthy Females from all racial groups with different social stratifications were involved. Female with a history of metabolic, endocrine, autoimmune, early menopause (<40 years), hepatitis, hepatoma and cirrhosis were not included in study. Female with an antiquity of medications such as calcitonin bisphosphonates, glucocorticoids, VD, calcium, anticonvulsants and hormone therapy were also not incorporated in study. Dual-energy X-ray absorptiometry (DEXA) scans were achieved with Hologic discovery (QDR sequences). Pictures were taken of the left hip and spine. BMD measurements were performed in grams / cm squared by taking the combined BMD number of two skeletal positions, for example the skeleton and spinal cord were marked and expressed as a T-score value.

Women were characterized based on the scale of bone mineral concentration; categorized into normal (T-score ≥ -1), osteopenic (T-score -1.1 to -2.4) and osteoporotic (T-score <-2.5) agreeing to WHO classification.

Venous blood sample was collected (2 milliliter) in Serum gel tube and serum was segregated after centrifugation and deposited.

VD was analysed in serum by using VD ELISA Kit (Cat# ab213966). The analytical understanding of the test was 1.98 ng/ml and recognition range was 0.5 ng/ml to 1010 ng/ml with inter and intra assay coefficient of variation (CV) of < 16% and < 4% individually. A VDBP level in serum was detected by using a commercially existing Human DBP ELISA Kit (Cat. No: 96577, Glory Science Co., Ltd.) by means of a recognition range of kit 8 µg/ml to 480 µg/ml.

Calcium was measured by Colorimetric Calcium Assay Kit (Cat# ab102505). The analytical sensitivity of the assay was 0.1mM and detection range was 0.1 mM to 25 mM. Phosphate was measured by Colorimetric Phosphate Assay Kit (Cat# ab65622). The analytical sensitivity of the assay was 1 µM and detection range was 0.001 mM to 1 mM.

Tumor Necrosis Factor was analysed by using Human (TNF)- ELISA Kit (Cat# E0082Hu). The analytical sensitivity of the assay was 1.52 ng/L and finding series was 3 ng/L to 900 ng/L with inter and intra assay coefficient of variation (CV) of <10% and

<5% correspondingly. Interleukin 6 was restrained by means of Human IL-6 ELISA Kit (Cat# E0090Hu). The analytical sensitivity of assay was 1.03 ng/L and recognition series was 2 ng/L to 600 ng/L with inter and intra assay coefficient of variation (CV) of <10% and <6% disparately.

Osteocalcin was estimated by using Human Osteocalcin ELISA Kit (Cat# ab270202). The investigative compassion of assay was 13.99 pg/ml and detection range was 52.08 pg/ml to 3333 pg/ml with inter and intra assay coefficient of variation (CV) of 7.1% and 6.1% consistently.

C-Terminal Telopeptide was measured by using Human C-peptide ELISA Kit (Cat# ab260064). The analytical sensitivity of assay was 1.45 pg/ml and detection range was 3.13 pg/ml to 200 pg/ml with inter and intra assay coefficient of variation (CV) of 3.7% and 2.6% separately.

Statistical Package for Social Sciences (SPSS) version 20 software was used. Descriptive statistics mean ± standard deviation was calculated. Analysis of Variance (ANOVA) test was completed to relate the alteration among groups and Spearman's correlation test was achieved to find association of study variables. Regression analysis was completed to predict the factors responsible for osteopenia /osteoporosis. A p-value of < 0.05 was measured as statistically important.

RESULT

A total number of 245 females were incorporated in the study. According to Dexa scores 105 (42.9%) were healthy females, 84 (34.3%) females were in osteopenia group and 56 (22.8 %) females were in osteoporosis group.

Table-I describes the descriptive appearances of the study population. The mean age and VDBP were found to be significantly different in all three groups (p<0.005), however, BMI was not meaningfully different among all groups. The mean serum values of VD, TNF α, IL-6 and CTX were found to be dissimilar in all three groups (p<0.005).

Fig.1 describes correlation of VD in all three groups, significant strong negative correlation of VD with age (r= -0.464, p<0.001). Correlation between VD and BMI was r= -0.189 (p<0.005). A strong positive correlation was observed amongst VD and VDBP (r= 0.596, p<0.001). A strong negative correlation was also observed among VD and TNF, IL-6, osteocalcin, and CTX respectively (p<0.001)

Table-II describes regression analysis between osteopenia and osteoporosis group after adjusting with risk factor such as BMI, VDBP, Ca, Phosphate, TNF α, IL-6, osteocalcin and CTX. It was observed that with every one unit rise in BMI the frequency of osteopenia is significantly reduced by 72% (p<0.05) and prevalence of osteoporosis is significantly decreased by 66.2% (p<0.05) and with every one unit intensification in VDBP levels the occurrence of osteoporosis is diminished by 56.4% (p<0.05).

DISCUSSION

Obesity is associated with VD deficiency, as increased adipose tissue occupies the circulating form of VD, thus establishing a reciprocal relationship between VD with BMI. BMI was not different in normal/osteopenic and osteoporotic groups.

BMD analysis provides a measure of bone strength, a low BMD measure is assessed to increase the risk of fracture but is not a marker of osteoporosis. Recognition of biomarkers associated with osteoporosis and osteoporosis will therefore play an important role in detecting these diseases at early stages.[10] In our study, a weak correlation was observed between BMI and

Table-I: Descriptive characteristics of study population.

Variables	*Normal N=105 Mean±SD*	*Osteopenia N=84 Mean±SD*	*Osteoporosis N=56 Mean±SD*	*p-value*
Age (Years)	38.07 ± 13.02	47.65 ± 9.71	48 ± 12.25	0.00
BMI (Kg/m^2)	25.44 ± 5.8	25.85 ± 5.12	25.44 ± 4.68	0.85
Vit D (25-OHD) (ng/ml)	26.5 ± 23.2	17.7 ± 16.63	19.89 ± 18.38	0.01
Vitamin D Binding Protein (VDBP) (µg/ml)	542.99 ± 626.45	406.09 ± 348.35	312.77 ± 167.38	0.01
Ca (mM)	7.73 ± 1.48	7.67 ± 1.35	7.82 ± 1.46	0.82
Phosphate (mM)	4.26 ± 0.98	4.49 ± 1.19	4.53 ± 0.94	0.19
Tumor Necrosis Factor (TNF) (ng/L)	34.16 ± 36.98	59.91 ± 36.54	43.51 ± 37.74	0.00
Interleukin-6 (ng/L)	4.61 ± 4.59	7.96 ± 5.28	5.85 ± 4.97	0.00
Osteocalcin (pg/ml)	16.27 ± 9.74	15.62 ± 11.79	15 ± 10.45	0.76
C-Terminal Telopeptide (CTX) (pg/ml)	13.63 ± 15.44	24.18 ± 19.78	19.63 ± 20.08	0.00

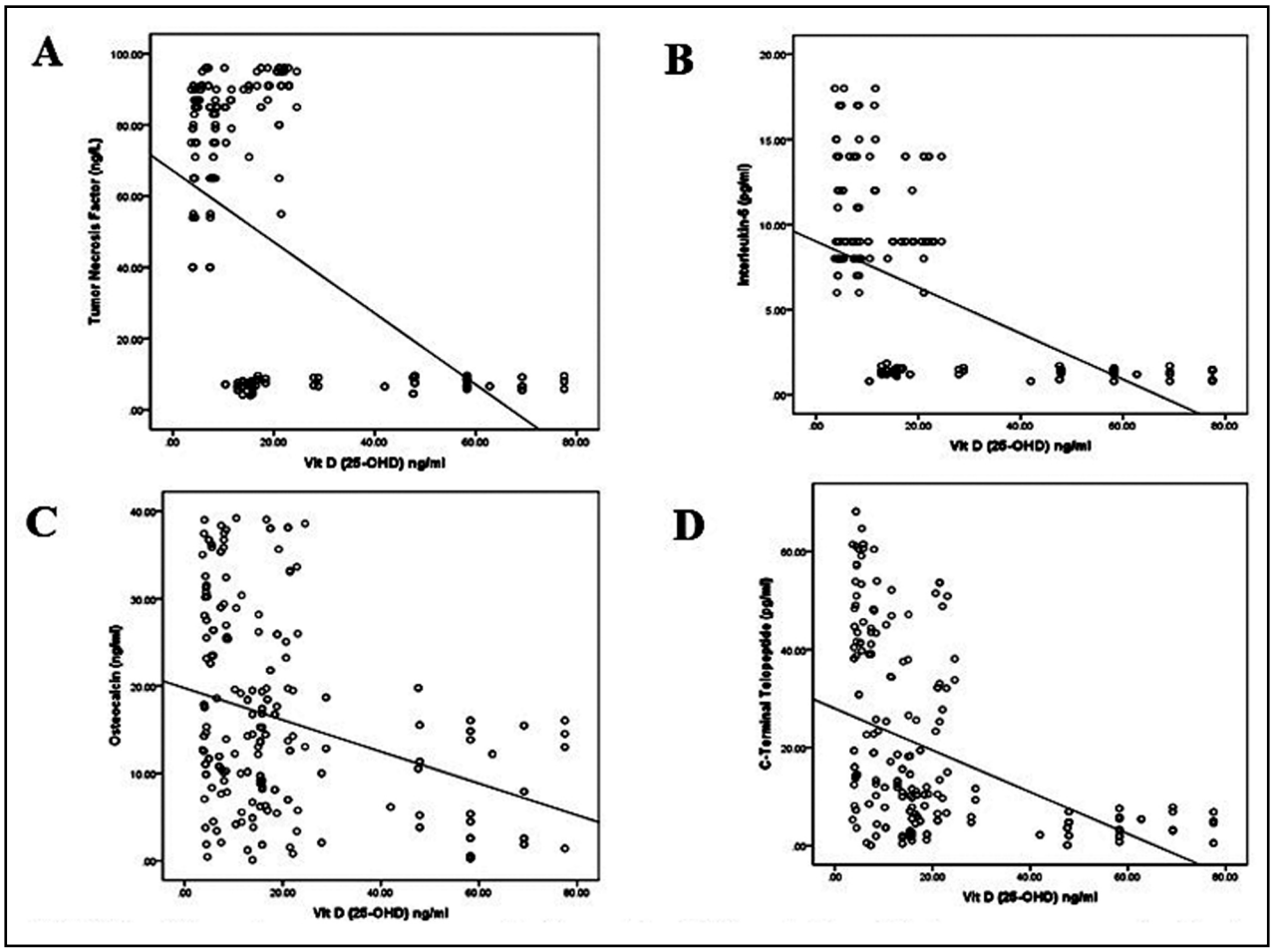

Fig.1: Correlation of Vitamin D with cytokines.

VD levels. An international study shows that BMI is significantly higher in individuals with insufficient and deficient serum VD levels.[11] The results of our study agree with previous study which showed similar association of BMI and VD.[12] Results from a bidirectional Mendelian randomization study suggested a causal association between higher BMI on decreased VD status. VD and BMI (12). A topical meta-analysis found a contrary association between vitamin D and percent fat mass.[13]

Regardless of the age group, the occurrence of VD

Table-II: Regression Analysis between osteopenia and osteoporosis.

Variables	*Osteopenia*		*Osteoporosis*	
	Beta	*p value*	*Beta*	*p value*
BMI (Kg/m^2)	0.280	0.027	0.338	0.039
VDBP (ug/ml)	0.200	0.268	0.436	0.007
Ca (mM)	-0.050	0.733	-0.117	0.446
Phosphate (mM)	-0.062	0.619	-0.017	0.909
Tumor Necrosis Factor (TNF) (ng/L)	0.070	0.600	0.082	0.551
Interleukin-6 (ng/L)	-0.086	0.695	-0.639	0.042
Osteocalcin (pg/ml)	-0.095	0.652	0.239	0.406
C-Terminal Telopeptide (CTX) (pg/ml)	-0.232	0.074	0.209	0.198

deficiency in obese people was 35% greater than in the normal weight group, and 24% raised in the overweight population. The main outcomes of our study were the greater frequency of osteoporosis in the age group of women (47.65 ± 9.71) and the change in osteoporosis in the age group of women (48 ± 12.25).

Additionally, in our research, we observed that there was a noteworthy alteration among VD levels in women with osteopenia is lower compared to the normal and osteoporotic group. A similar study regarding VD and BMD in premenopausal and postmenopausal women reported similar results.[14] It has been observed that low levels of VD have been associated with women of childbearing age and postmenopausal women by diminutive levels of BMD. This may have been due to little calcium consumption and insufficient sun exposure in older women, leading to VD deficiency and reduced absorption of calcium.[15] Different calcium, serum magnesium levels were not meaningfully changed among the three groups in our study which are similar to certain earlier results[16] and is consistent with an additional study that exhibited reduced serum levels of magnesium in osteoporotic women related to the normal group.[17]

Our study shows significant findings of VDBP, indicating that it is lower in osteoporotic females compared to osteopenic and normal group. Other studies have revealed that serum VDBP levels are significantly reduced in women presenting with fracture and low BMD.[18] VDBP can be used for early recognition and diagnosis of bone diseases such as osteopenia and osteoporosis, providing an essential time window for treating and preventing severe symptoms such as fractures.[19]

Particular cytokine levels in bone homeostasis show an important part of regulating osteoclast action, so an understanding of cytokines can offer responsiveness within usual calcium homeostasis and the nature of bone changes such as bone and marrow transplantation.[17] Osteopenic women have been observed to have greater levels of TNF-α in osteoporotic compared to normal women, supporting that TNF-α is an important source of bone resorption.[20] It raises osteoclast variation and prevents osteoblast existence, thus reducing BMD.[21] In addition, CTX reduction in bone resorption decreased because of induction of TNF-α, which occurs owing to decreased collagen deprivation. The aim of treatment in osteopenia and osteoporosis via improved interleukin in osteopenic women was demonstrated by the upturn in IL-6 in this research, which is similar to the research by Green et al.[22] (in our study, higher levels of TNF-α and IL-6 was found in osteopenic women), suggesting the IL-6 and TNF-α play important role in bone regeneration and development methods. In one study, women were osteopenic in both pre- and post and postmenopausal groups,[23] and there was an important negative association of TNF-α through BMD in premenopausal women. Estrogen and ovarian insufficiency in postmenopausal women require macrophage colony-stimulating factor (mCSF)-stimulated pro-inflammatory cytokines series signaling, gene expression cascade and IL-6.[24]

Recent literature recommends that exogenous TNF-α increases endoplasmic reticulum irritation, losses collagen oozing promotes apoptosis, and induces a rise in bone turnover regardless of reason of decreased bone microarchitecture. Therefore, it increases possibility of fracture independent of slight BMD.[14] Osteocalcin and CTX levels were elevated in osteopenic women in our study, which is reinforced by one more study.[25]

Conclusion: BMI and VDBP levels were found to be predictive factors for osteopenia and osteoporosis in studied population. These findings emphasize on the importance of monitoring Vitamin D levels, BMI, and VDBP to assess bone health and predict the risk of osteoporosis and osteopenia in the female population of Karachi, Pakistan. Additionally, strategies aimed at maintaining optimal Vitamin D levels and managing BMI may help to mitigate the risk of these conditions.

Recommendations: A comprehensive assessment of inflammatory biomarkers and associated metabolic pathways may help identify bone functions and predict the subacute phase of fractures, which may help reduce bone damage, preserve bone density, and reduce the risk of secondary fractures. This information can perhaps be used to design policies and initiate educational programs to increase the population's awareness about making lifestyle modifications such as regular physical exercise and improving the diet to avoid vitamins and mineral deficiencies.

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Authors Contribution:

AA, reviewed, edited, and supervised manuscripts.
HS, AK, RS did manuscript writing
MA did a manuscript statistical analysis.
RR conceived, designed, and supervised the manuscript.