**RESEARCH ARTICLE**

## Relation of Vitamin D with Bone Mineral Density and Bone Turnover Markers in Healthy Saudi Men.

**Alhazmi, A., S.**
Assistant professor of clinical biochemistry, faculty of applied medical science, Taif University.

| *Manuscript Info* | *Abstract* |
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| | Vitamin D is a fat-soluble vitamin that have an important biological function in maintaining calcium homeostasis and normal bone growth. Subject and methods: 346 healthy Saudi men recruited into the center of excellence for osteoporosis research and classified according to vitamin D status. For each, BMD for lumbar spine and femoral necks were measured. Also, biochemical markers include serum calcium, phosphorus, ALP, iPTH, 25(OH) vitamin D, osteocalcin, PINP, CTX, and NTX were estimated. Results: Normal vitamin D level represent about 16.18% of our subjects while the remainder were vitamin D deficient 83.82 %. There were significant differences in iPTH, BMD of the lumbar spine, and both femoral necks, CTX, and NTX between four groups (P<0.01). Also, 25(OH) vitamin D levels showed a negative correlation with iPTH and NTX levels, while the positive correlation with BMD of both femoral necks, and PINP level (P<0.01). Conclusion: Low vitamin D level will be associated with high PTH level that may activate bone turnover and decrease BMD leading to increasing bone susceptibility to fracture. |

## Introduction:-

Osteoporosis is a common, costly public health problem, and its prevention is a great importance. Low bone mineral density (BMD) is the main factor associated with bone fractures (El Maghraoui *et al.,* 2009). Vitamin D is an important nutrient for normal bone growth and maintenance (Viljakainen *et al.,* 2006). It has an important role in calcium homeostasis. The active form of vitamin D called 1,25-dihydroxyvitamin D (1,25(OH) vitamin D) promote calcium homeostasis through its actions on the bone and intestine. The 1,25(OH) vitamin D formed in the kidney stimulates intestinal calcium absorption. In the bone, it enhances the release of calcium and phosphorus from stores by induction of stem cell monocytes to differentiate into mature osteoclasts, which stimulating bone resorption followed by the release of minerals into circulation. The level of vitamin D is regulated through the interaction of a number of factors include, serum parathyroid hormone (PTH), calcium levels, intestinal absorption, and renal activation of 25 (OH) vitamin D into 1,25 (OH) vitamin D (Boot *et al.,* 2011). The main sources of vitamin D are dietary intake and exposure to ultraviolet light from sunlight. The vitamin D deficiency is a predominant problem in many populations (Holick, 2007). Its deficiency is diagnosed by 25 (OH) vitamin D measurement. In Saudi Arabia, a plentiful sunny area, several studies done on men and women at different ages showed a high prevalence of vitamin D deficiency among this population. The recent study was done on healthy adult men found a high prevalence 98% of study subjects having a vitamin D deficiency (Alharbi, 2011). Vitamin D is considered deficient, when serum 25 (OH) vitamin D value is < 20 ng/mL; insufficient when serum value is 20-39 ng/mL and normal when serum level is > 39 ng/mL (Von Domarus *et al.,* 2011). BMD is the gold standard technique for osteoporosis diagnosis (Bischoff-Ferrari *et al.,* 2004). Vitamin D deficiency can be an important factor for the development of osteoporosis (Lips *et al.,* 2006). Chronic deficiency or insufficiency of 25 (OH) vitamin D impair calcium absorption. Low serum calcium level triggers elevation of PTH, which called secondary hyperparathyroidism. Elevated PTH, leading to increasing bone turnover, bone loss, and finally development of osteoporosis and bone fracture risk (Audran and Briot, 2010). Several previous studies found that low 25 (OH) vitamin D levels are common among women with osteoporosis (Steingrimsdottir *et al.,* 2005). One of this studies concludes that,

insufficient serum 25 (OH) vitamin D concentrations are associated with lower BMD at the lumbar spine, and whole body and its effect was greater than the physical activity (George, 2009). In animal models, treatment with vitamin D increases bone formation and the number of osteoblasts and osteoblast precursor cells. Modern biochemical bone turnover markers helped increase our understanding of the bone remodeling cycle, skeletal disorders pathogenesis, and the response to the therapy. Moreover, they can be used in the assessment of the balance between bone resorption and formation, and can measure changes occur in the bones. Bone turnover markers used to be an additional tool with BMD to the assessment of the risk of fracture. *In vitro* studies found a positive effects of 1,25(OH) vitamin D on mRNA, protein expression including osteocalcin (most abundant noncollagenous bone matrix protein), collagen type I (major bone matrix protein), and alkaline phosphatase (ALP) activity by using osteoblast-like cell line (Hill et al., 2006; Burge *et al.,* 2007). The present study was aimed to explore the association between serum 25 (OH) vitamin D concentration and BMD of the lumbar spine, and right and left femoral neck in one hand and bone turnover markers in other.

## Subjects and Methods:-
### Subjects and questionnaire:-
The present study was done upon 346 healthy Saudi men recruited randomly, aged between 20-50 years. For each, the biochemical markers for liver, kidney, and some endocrine glands functions were investigated to make sure any individual included in this project was healthy. A standard questionnaire about Age, body weight, height, BMI, presence of some chronic diseases (such as diabetes mellitus and hypertension), lifestyle, was used. Also, the use of vitamins and medications were included in this questionnaire to make sure any individual included was not used any medication affect calcium and vitamin D metabolism.

### BMD measurement:-
BMD (g/cm2) of the anteroposteriorly lumbar spine (L1-L4), and both right and left necks of femur were determined by using dual-energy X-ray absorptiometry (DXA, LUNAR Model, USA).  T-score for each BMD calculated as:
T-score =(Patient`s BMD − young adult mean BMD) /1st SD of young adult BMD. Based on WHO criteria, all subjects with T-score < −2.5 were diagnosed as osteoporotic patients while T-score between −1 and −2.5 classified as having osteopenia and a T-score > −1 is considered normal (World Health Organization, 1995).

### Biochemical parameters measurement:-
Ten mL blood was collected from overnight fasting individuals (10-12 hours) in plain tube for calcium, phosphorus, ALP, intact PTH, 25 (OH) vitamin D, and bone turnover markers including bone formation (osteocalcin and PINP) and bone resorption (CTX and NTX) under standardized condition. Immediately, the sample was centrifuged after collection, and the serum was stored at 20 °C. Calcium, ALP, and phosphorus levels were measured using VITROS 250 Chemistry autoanalyzer (15). The intact PTH was measured by direct sandwich chemiluminescence immunoassays using LIASON autoanalyzer (Ersfeld *et al.,* 2004). Four bone turnover markers (osteocalcin, PINP, CTX, and NTX) were measured using immunoassay by Cobas e autoanalyzer (Roche Diagnostics GmbH, D-68298 Mannheim, Germany) (16). Direct competitive chemiluminescence immunoassay was used for serum 25(OH) vitamin D measurement (Liason Diasorin) (Ardawi *et al.,* 2010). Individuals included in our project were classified according to serum 25(OH) vitamin D level into: class I severe deficient subjects that serum vitamin D level was less than 12.5 nmol/l; class II with moderate deficiency of serum vitamin D level ranged between 12.5-25 nmol/l; class III mild deficiency of serum vitamin D ranged between 25-50 nmol/l; class IV insufficient vitamin D ranged between 50-75 nmol/l; and class V sufficient vitamin D level more than 75 nmol/l (Kevin, 2011).

### Statistical analysis:-
SPSS software version 16 (SPSS Inc., Chicago, IL, USA) was used in the performance of statistical analysis. The correlations were tested by using Spearman`s test. ANOVA was used in comparisons performance. Both comparisons and correlations were considered statistically significant when P < 0.05.

## Results:-
The study was carried out on 346 healthy Saudi men, classified according to serum 25 (OH) vitamin D levels. Normal vitamin D level represent about 16.18% of our subjects while the remainder were vitamin D deficient (83.82 %). Vitamin D deficient subjects were subclassified into; mild deficient subject contribute about 26.89% of deficient individuals, moderate deficient 52.75%, and severe deficient 30.63%. Table 1 represent age, BMI, serum calcium,

phosphorus, ALP, intact PTH, BMD of lumbar spine (L1-L4) and of right and left femoral neck, osteocalcin, PINP, CTX and NTX of each group. A comparison by using ANOVA showed a significant difference in intact PTH level between normal (4.95 pmol/L), mild (5.60 pmol/L), moderate (6.22 pmol/L) and severe (7.52 pmol/L) deficient vitamin D individuals. In addition, there is a significant difference in BMD of lumbar spine, right and left femoral neck between normal (1166.8, 1123.3 and 1120.3), mild (1145.3, 1056.6 and 1064,1), moderate (1075.5, 966.9 and 965.0) and severe (1033.0, 941.2 and 932.1) vitamin deficient individuals respectively. Also there are significant differences in bone turnover markers; CTX and NTX between normal (431.5 and 658.3), mild (512.5 and 708.6), moderate (548.4 and 903.8) and severe (643.2 and 954.8) vitamin D deficient individuals respectively. Table 2 represent a correlation between all measured parameters by using Pearson`s correlation coefficient. The age showed a positive correlation with only serum intact PTH, while negative correlation with BMD of lumbar spine and right and left femoral neck, serum calcium, phosphorus, ALP, vitamin D, osteocalcin, PINP, CTX and NTX ($P < 0.01$). Serum calcium level showed a positive correlation with BMD of the right and left femoral neck and bone formation marker (osteocalcin and PINP) ($P<0.01$). Moreover, phosphorus level showed a positive correlation with bone formation marker (osteocalcin and PINP) and only NTX a bone resorption marker ($P<0.01$). The intact PTH level showed a positive correlation with bone resorption marker (NTX) and negative correlation with bone formation marker (osteocalcin) and BMD of right and left femoral neck ($P<0.01$). Finally, serum vitamin D level showed a positive correlation with BMD of the right and left femoral neck and bone formation marker (PINP) while a negative correlation with bone resorption marker (NTX) ($P<0.01$).

**Table1: Age, BMI, s-Ca, s-PO4, s-ALP, s-iPTH, BMD of lumbar spine, and right and left femoral necks, osteocalcin, PINP, CTX, and NTX in severe, moderate, and mild deficient and sufficient individual expressed as (Mean±SD) and comparison by using ANOVA**

|  | Severe deficient N= 59 (17.05%) | Moderate deficient N=153 (44.21%) | Mild deficient N=78 (22.54%) | Sufficient N=56 (16.18%) | P. value |
|---|---|---|---|---|---|
| Age (years) (mean±SD) | 34.23 ± 1.57 | 35.61 ± 0.68 | 35.63 ± 1.09 | 39.00 ± 4.11 | |
| BMI (mean±SD) | 27.78 ±0.81 | 27.86 ± 0.35 | 28.20 ± 0.49 | 27.30 ± 1.82 | |
| s-Ca (mmol/L) (mean±SD) | 2.40 ± 0.02 | 2.39 ± 0.01 | 2.40 ± 0.01 | 2.39 ± 0.05 | |
| s-PO$_4$ (mmol/L) (mean±SD) | 1.23 ± 0.02 | 1.24 ± 0.01 | 1.24 ± 0.02 | 1.26 ± 0.06 | |
| s-ALP (U/L) (mean±SD) | 82.79 ± 3.15 | 75.65 ± 1.40 | 77.01 ± 2.10 | 75.00 ± 9.05 | |
| s-iPTH(pmol/L) (mean±SD) | 7.52 ± 0.39 | 6.22 ± 0.18 | 5.60 ± 0.22 | 4.95 ± 0.93 | ** 0.00 |
| BMD of L1-L4 (mean±SD) | 1033.0±24.76 | 1075.5±9.78 | 1145.3±13.01 | 1166.8±20.20 | ** 0.00 |
| BMD of right femoral neck (mean±SD) | 941.2±23.21 | 966.9±9.58 | 1056.6±12.03 | 1123.3±24.33 | ** 0.00 |
| BMD of left femoral neck (mean±SD) | 932.1±23.52 | 965.0±9.73 | 1064.1±12.59 | 1120.3±35.56 | ** 0.00 |
| Osteocalcin (mean±SD) | 27.13±1.54 | 27.54±1.01 | 28.51±1.44 | 20.12±2.40 | |
| PINP (mean±SD) | 71.63±5.23 | 66.45±3.11 | 67.21±4.16 | 45.43±9.93 | |
| CTX (mean±SD) | 643.23±40.58 | 548.42±16.93 | 512.52±28.58 | 431.50±31.33 | * 0.02 |
| NTX (mean±SD) | 954.85±83.77 | 903.83±34.81 | 708.60±35.41 | 658.33±137.70 | ** 0.004 |

* $P< 0.05$ is considered significant **$P< 0.01$ is considered highly significant

**Table 2: Correlation between age, BMI, BMD values at lumbar spine (L1-L4) and at right and left femoral neck, s-Ca, s-PO₄, s-ALP, s-iPTH, s-25OHD₃, s-OC, s-PINP, s-CTX, and s-NTX in all individuals by using Pearson`s correlation coefficient.**

| Variable | Age | BMI | BMD1 | BMD2 | BMD3 | Ca | PO$_4$ | ALP | PTH | Vit-D | OC | PINP | CTX | NTX |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | | ** 0.38 | | ** -0.27 | ** -0.27 | ** -0.32 | ** -0.17 | ** -0.15 | ** 0.21 | | ** -0.48 | ** -0.55 | ** -0.45 | ** -0.15 |
| BMI | | | ** 0.20 | ** 0.16 | ** 0.15 | ** -0.19 | | | * 0.13 | | ** -0.33 | ** -0.32 | ** -0.28 | ** -0.16 |
| BMD1 | | | | ** 0.62 | ** 0.62 | | | | | | | | | ** -0.27 |
| BMD2 | | | | | ** 0.96 | * 0.11 | | | ** -0.11 | ** 0.14 | | ** 0.16 | | ** -0.18 |
| BMD3 | | | | | | * 0.12 | | | ** -0.14 | ** 0.15 | | ** 0.17 | | ** -0.23 |
| Ca | | | | | | | ** 0.18 | * 0.12 | | | | * 0.15 | * 0.14 | |
| PO$_4$ | | | | | | | | | * -0.13 | | ** 0.20 | ** 0.20 | | * 0.12 |
| ALP | | | | | | | | | | | ** 0.24 | ** 0.31 | ** 0.19 | |
| PTH | | | | | | | | | | ** - 0.216 | ** -0.22 | | | ** 0.15 |
| Vit-D | | | | | | | | | | | | ** 0.15 | | ** -0.21 |

BMI, BMD1 (bone mass density of lumbar spine), BMD2 ( bone mass density of right femoral neck), BMD3 (bone mass density of left femoral neck),  Ca, PO₄, ALP, iPTH, vit-D, OC, PINP), CTX, NTX.

 ** Correlation is significant at 0.01 level                    * Correlation is significant at 0.05 level.

## Discussion:-

Vitamin D is a fat-soluble vitamin important for the maintenance of bone health. The major source of vitamin D is the synthesis of vitamin D from steroid precursors called 7-dehydrocholesterol (7-DHC) in the skin by UV action (Perez-Lopez, 2007). Several environmental factors can affect the skin's production of vitamin D, including skin pigmentation, sunscreen use, clothing, latitude, season, and time of day. The remainder source for vitamin D is dietary intake (Holick and Garabedian, 2006). Vitamin D deficiency still to be an epidemic problem in many countries around the world. Several studies done on Saudi women showed a high prevalence of vitamin D deficiency in these subjects despite Saudi Arabia is a sunny area. **Alharbi *et al.,* 2013** reported vitamin D deficiency were 98.8% for their study population. Nearly 12.34% of vitamin D deficiency men had severe vitamin D deficiency while 61.58% and 24.88% had moderate and mild deficiency respectively. Moreover, other study was done by **Mir Sadat *et al.,* 2011** on Saudi men, reported about 37% of their study population had vitamin D deficiency while **Naeem *et al.,* 2011** reported about 67.9%. Moreover, **Al-Elq** reported a high prevalence of vitamin D deficiency about 100% in Saudi medical students (male and female) (Al-Elq, 2013). Our study found that vitamin D deficiency is present in about 83.82% of our subjects. Serum vitamin D was reported as an independent determinant of whole body BMD (Bischoff-Ferrari et al., 2005). Vitamin D is important in calcium absorption and maintenance of bone health. In individual with vitamin D deficiency, only 15% of dietary calcium is absorbed leading to low serum calcium levels that will be increased PTH level as a compensatory mechanism to maintain normal blood calcium level (Holick and Garabedian, 2006). Several studies found a negative correlation between 25(OH) vitamin D and iPTH level. **Ebeling *et al.,*** and **Pattanaungkul** studies, found a negative correlation between 25(OH) vitamin D level and iPTH. Our result is in agreement with both previous studies. Increase PTH levels due to low calcium level called secondary hyperparathyroidism that stimulates bone resorption to maintain normal blood calcium level. The BMD that measures the quantity of the calcified bone still until now the best standard technique for the diagnosis of osteopenia and osteoporosis. The long-term bone resorption may lead to low BMD and increase susceptibility to bone fractures. A study was done by **Minna *et al.,* 2012** found that, insufficient serum 25 (OH) vitamin D levels were associated with lower BMD at the lumbar spine, and the right and left femoral neck bones. In accordance with

these results, our results showed low BMD of the lumbar spine, and the right and left femoral neck in all vitamin D deficient men groups compared with sufficient individuals with lower levels in severe vitamin D deficient group. Previous studies talk about the correlation between 25(OH) vitamin D level and the BMD in different populations. **Nakamura et al.,** study that done on Japanese women found a positive correlation between 25(OH) vitamin D level and the BMD of the femur, but not with lumbar spine BMD. Other study done on 220 Finnish young men showed a positive correlation between serum 25 (OH) vitamin D right and left femoral neck BMD. In Arab area, same results were found by **Arabi et al.,** a study done on Lebanese population. In agreement with previous studies, our results showed a positive correlation between 25(OH) vitamin D and BMD of right and left femoral neck but not with lumbar spine BMD. False elevation in lumbar spine BMD may due to degenerative change and aortic calcification associated with aging that may lead into a non-specific association between serum 25 (OH) vitamin D and BMD in this site. In addition, we know that iPTH mediate cortical bone resorption than trabecular bone and femur contain more cortical bone than trabecular bone. So, elevated iPTH secondary to low 25-(OH) vitamin D level may affect femoral bone than lumbar spine. However, no association between 25 (OH) vitamin D level and BMD has been found in other studies (panah et al., 2008, Tsai et al., 1997). In recent years, bone turnover markers have been shown to provide information about bone metabolism and pathophysiology of metabolic bone diseases such as osteoporosis. There are useful parameters in risk evaluation and monitoring of osteoporosis treatment (Denhardt et al., 2001). Several studies talk about consequences of vitamin D deficiency that include secondary hyperparathyroidism, accelerated bone loss, increase bone turnover, osteoporosis and finally bone fractures. Recently, a number of studies aimed to evaluate the relation between vitamin D deficiency and bone turnover markers. Bone turnover markers can be either bone formation or bone resorption markers. Bone formation markers either enzymes such as ALP, proteins such as osteocalcin secreted by osteoblast, or byproducts of type I collagen such as procollagen type 1 N-terminal peptide (PINP). Bone resorption markers are degradation products of bone collagen such as CTX and NTX (Al-Daghri et al., 2012). Bone turnover markers showed a gender variation with a higher level in male more than female, so our study was done upon only male subjects to overcome gender variation. The osteoblast is a prominent source of osteocalcin which comprises approximately 2% of bone non-collagenous proteins. Osteocalcin induction in response to 1,25-(OH)-vitamin D in osteoblasts will modulate bone mineralization (Nasser et al., 2013). A recent study was done by **Lu et al.,** found a significant inverse correlation between the bone turnover markers and serum 25 (OH) vitamin D levels (Nasser et al., 2013). Moreover, **Han-Kui** suggests that vitamin D insufficiency may be related to accelerated bone turnover and subsequently osteoporosis. The previous study done on Saudi male conclude that deficiency of 25 (OH) vitamin D can affect bone turnover markers (Ardawi et al., 2012). Our results showed a significant variation in bone resorption markers (CTX and NTX) between sufficient and vitamin deficiency groups with a higher level in severe deficiency group. Both age and BMI showed a negative correlation with bone turnover markers. The BMD in lumbar spine and right and left femoral neck showed a positive correlation with PINP and negative correlation with NTX. The serum iPTH level was positively correlated with NTX and negatively with osteocalcin. This may explain why increased PTH associated with increased bone fragility and increase susceptibility to fracture. Finally, vitamin D level showed a positive correlation with PINP while a negative correlation with NTX. All previous result support the fact that said vitamin D improve bone strength and maintain bone health.

## Recommendation and conclusion:-
Our study recommends the importance of vitamin D supplementation to maintain its normal blood level and bone health. In addition, normal blood vitamin D level maintain healthy bone and prevent the development of bone fragility and fracture. In conclusion, low vitamin D level will be associated with high PTH level that may activate bone turnover and decrease BMD, leading to increasing bone susceptibility to fracture.

## Acknowledgments:-

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