Relation of Vitamin B12, Folate, and Methylenetetrahydrofolate Reductase Polymorphism to Bone Mass Density in Healthy Saudi Men

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Abstract: Osteoporosis is a global health problem. The magnitude of the disease become larger in the Middle East region than in western countries. Local clinical observations and research data showed that osteoporosis is a common disorder in the Saudi population. Most of these observations were concentrating upon postmenopausal women, but other observations suggested that the disease could affect men to a comparable degree with women. The nutritional deficiencies play important role in osteoporosis development. So our project focused on the relationship between vitamin B12 and folate status with the BMD in healthy Saudi men.**Method**: Our study was consisted of 315 Saudi men. Based in WHO criteria they were classified according to T-score of their BMD of the anteroposterior lumbar spine (L1-L4), and right and left femoral neck into normal (n=235), osteopenic (n=70), and osteoporotic (n=10). The serum Ca, vitamin B12, folate, homocysteine, OC, PINP, CTx, and NTx levels were measured for each individual. Also MTHFR C677T genotype was performed for each individual to detect the relation of these different genotypes with BMD. **Results**: By using ANOVA serum vitamin B12 and folate levels were significantly reduced in osteopenic and osteoporotic patients compared with normal subjects (P<0.05), while serum homocysteine, NTx, and CTx levels were significantly elevated in osteopenic and osteoporotic compared with the normal individuals (P<0.05). Serum vitamin B12 level has a significant negative correlation with serum homocysteine, CTx, and NTx levels (P<0.01) by using Pearson`s correlation coefficient. In addition, serum folate has a significant positive correlation with BMD of right and left femoral neck, and serum Ca levels (P <0.01), while serum homocysteine showed a significant negative correlations with BMD of lumbar spine (L2-L4) and right and left femoral neck (P <0.01). Serum OC, PINP, CTx, and NTx levels were inversely correlated with age and BMI (P <0.01). Homozygous (T677T) was associated with elevated blood homocystiene level compared with wild one. **Conclusion**: Elevated blood homocysteine level show to play a role in reduction of BMD Lumbar spine (L1-L4) and right and left femoral neck. Vitamin B12 and folate status and MTHFR C677T polymorphism may maintain BMD through their effect on blood homocysteine level.
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1. Introduction

Osteoporosis is a major health problem that is characterized by low bone mineral density, deterioration of bone microarchitecture, and an increased risk of fracture **(El maghraoui *et al.*, 2012)**. Osteoporotic fractures affect the quality of life and are associated with premature mortality **(El maghraoui *et al.*, 2012)**. Approximately 40-50% of women sustain osteoporotic fractures in their lifetime so that studies initially focused upon females. But several epidemiologic studies showed that, 25-33% of men in some populations suffer from osteoporotic fractures in their lifetime. Several factors influence the development of osteoporosis in men including genetic factors, sex hormones mainly estrogen, hyperparathyroidism, hyperthyroidism, lifestyle (such as smoking and alcohol consumption), and finally nutritional deficiencies (such as Ca and vitamins) **(Kanis *et al.*, 2002; Hodgeson *et al.*, 2003).**

Previously, some epidemiologic investigations suggest that increased plasma homocysteine levels could be associated with osteoporotic fractures **(Van Meurs *et al.*, 2004; Sato *et al.*, 2005)**. Homocystinuria, a rare autosomal recessive disease characterized by markedly elevated of plasma homocysteine levels, has several clinical manifestations including early onset of generalized osteoporosis. In addition, *in vivo* and *in vitro* studies suggest that collagen cross-linking in bone is impaired in homocystinuria patients, and also osteoclast stimulation is involved **(Abdellah *et al.*, 2012; Ivana *et al.*, 2012)**. Also, a mild elevation in plasma homocysteine level, termed hyperhomocysteinemia, is associated with age-dependent bone loss **(Seshadri *et al.*, 2002)**. Moreover, hyperhomocysteinemia seems to be associated with higher circulating concentrations of bone turnover markers such as osteocalcin (OC), Procollagen type I propeptides (PINP), and Telopeptides of type I collagen (CTx and NTx), suggesting a disturbance of

bone metabolism. In animal studies, chicks fed a homocysteine supplemented diet had altered bone growth, bone matrix, and bone composition when compared with control chicks **(Miyao *et al.*, 2000)**. The folate and vitamin B12 are important cofactors in homocysteine metabolism, and low status of these two vitamins is the primary determinant of elevated plasma homocysteine levels in elders **(Naharaci *et al.*, 2012)**. Several studies suggest that, both lower dietary intakes and blood levels of folate and vitamin B12 may be associated with decreased BMD, greater bone loss, and higher risk of osteoporotic fracture **(Gerdhem *et al.*, 2007)**. Moreover, *in vitro* studies indicate that low folate and vitamin B12 concentrations promote osteoclast activity and bone resorption, whereas elevated concentrations may stimulate bone formation through its effect on osteoblast activity (**Haroon *et al.*, 2012; Hermann *et al.*, 2007)**.

5,10 Methylenetetrahydrofolate reductase (MTHFR) is important enzyme in homocystiene metabolism and play a role in bone health. A number of studies have investigated the role of the C677T MTHFR polymorphism on bone phenotypes in older men and women. A point mutation in the MTHFR gene (C677T), which induces a substitution of valine for alanine, is a common variant, which is associated with reduced enzyme activity and with increased homocysteine levels. Some studies reported this polymorphism to be associated with either an increased risk of osteoporotic fractures **(Bathum *et al.*, 2004; Villadsen *et al.*, 2005)** or lower BMD **(Abrahamsen *et al.*, 2006)**, but other studies fail to confirm this association **(Wang and Liu, 2011)**.

Day to day clinical observations as well as the available research data in Saudi Arabia showed that osteoporosis is a common disorder in the Saudi population. Most of the previous work done in the kingdom was concentrating upon postmenopausal women. The aim of our study is to evaluate the relationship between vitamin B12 and folate status with BMD values and osteoporosis susceptibility in healthy Saudi men. In addition, to determine the MTHFR C677T genotypes in a sample of healthy Saudi men and its relation to homocysteine levels and bone health.

2. Subjects and Methods:

The calculated sample size was consisted of 315 healthy Saudi males who were lived in Jeddah and visit the Centre of Excellence for Osteoporosis Research (CEOR) in King Fahad Medical Research Center in King Abdulaziz university over a 12 months period from September 2010 to September 2011, recruited in random, aged between 20-50 years. For each individual, the biochemical markers for liver, kidney, and some endocrinal glands functions were investigated to make sure any individual included in this project was healthy. A standard CEOR questionnaire was used to collect information from each subject about Age, body weight, height, BMI, medical history about the presence of some chronic diseases (such as diabetes mellitus and hypertension), lifestyle, smoking habits, coffee and tea consumption and the use of vitamins and medications to make sure any individual included in this project was not used any medication that may affect Ca, vitamin B12, folate, and homocysteine levels.

BMD measurement:

For each individual the BMD (g/cm2) of the anteroposterior lumbar spine (L1-L4), and right and left femoral neck were determined by using dual-energy X-ray absorptiometry (DXA) using (LUNAR Prodigy Model, USA), and the T-score was calculated [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:

Blood from overnight fasting subjects (10-12 hours) were collected under standardized condition in different tubes: in EDTA containing tube for complete blood count (CBC) to exclude any anaemic patients and for MTHFR genotypes determination, while blood collected in plain tube was used for calcium, phosphate, folate, vitamin B12, bone turnover markers (OC, PINP, CTx and NTx) and homocysteine estimation. Sample was centrifuged immediately after collection and the serum separated in a number of Eppendorf tube. Each Eppendorf tube were refrigerated at -80 ^{0}C until analysis. The vitamin B12 and folate levels were determined by using a competitive electrochemiluminescence assay (**Elecsys, 2010**), while homocysteine was determined by using the VITROS homocysteine Slide method (VITROS 250 Chemistry System Autoanalyzer- Ortho-Clinical Diagnostics-Johnson & Johnson Co., USA). Bone turnover makrers were measured using Cobas e immunoassay analyzer (Roche Diagnostics GmbH, D-68298 Mannheim, Germany) **(Ardawi *et al.*, 2010)**.

MTHFR genotyping:

The DNA was extracted from blood in EDTA containing tube, then the MTHFR C677T genotyping was performed by PCR with the primers 5′-CAA AGG CCA CCC CGA AGC -3′ (forward) and 5′-AGG ACG GTG CGG TGA GAG TG-3′ (reverse). Samples were amplified for 35 cycles consisting of denaturation at 94 °C for 30 seconds, annealing at 59 °C for 45 seconds, and extension at 68 °C for 45 seconds; and then followed by a final extension step at 68 °C of 7 min. Since the C-to-T transition at nucleotide 677 produces a Hinf I digestion site, the endonuclease can cleave the

amplified product derived from the mutant restriction site into 175-bp and 70-bp fragments, and leaves the wild-type gene unaffected. The digested products were separated electrophoretically on 3% agarose gels **(Wang and Liu, 2011)**.

3. Results:

The study was carried out on 315 healthy Saudi men classified according to BMD values at lumbar spine (L1-L4) and right and left femoral neck into three groups, the normal group (T-score <-1) (n = 235), osteopenic (-2.5 <T-score> -1) (n = 72), and osteoporotic (T-score<-2.5) (n = 8). Table 1 represent a comparison of age, BMI, BMD values at the lumbar spine (L1-L4) and right and left femoral neck, s-Ca, s-Vitamin B12, s-folate, s-homocysteine, s-OC, s-PINP, s-CTx, and s-NTx for the normal, osteopenic, and osteoporotic groups by using ANOVA. A significant differences (P<0.05) were showed in age and BMI in normal (34.88±9.33; 28.44±4.72), osteopenic (36.96± 10.07; 26.64±4.71), and osteoporotic groups (41.64±7.43; 25.05±3.50). Also, our parameters of interest s-vitamin B12, folate, and homocysteine were showed a significant differences in normal (338.8±106.4; 12.29±3.85; and 10.97±3.19), osteopenic (215.15±84.03; 7.59±1.49; and 12.29±3.12), and osteoporotic (250.18±19.22; 6.6±4.64; and 13.90±4.02 (P<0.05). Only the bone resorption markers s-CTx and s-NTx but not bone formation markers were showed a significant differences (P<0.05) between normal (508.15±195.58; 741.51±267.20), osteopenic (603.88±275.64; 1177.80±717.34), and osteoporotic (674.45±143.36; 290.50±200.48) groups.

Table 2 represent a correlation by using Pearson`s correlation coefficient between age, BMI, BMD values at the lumbar spine (L1-L4) and right and left femoral neck, s-Ca, s-Vitamin B12, s-folate, s-homocysteine, s-OC, s-PINP, s-CTx, and s-NTx in the entire studied groups (n = 315). There was a significant positive correlation between age and BMI (r = 0.38), while a significant negative correlation between age and BMD values at right and left femoral neck (r = -0.27), s-Ca (r = -0.26), s-OC (r = -0.48), s-PINP (r = -0.55), s-CTx (r = -0.44), and s-NTx (r = -0.17) was determined. In relation with BMI there was a significant positive correlation with BMD values at lumbar spine (L1-L4) (r = 0.20) and right and left femoral neck (r = 0.15), while a significant negative correlation with s-OC and s-PINP (r = -0.33), s-CTx (r = -0.27), and s-NTx (r = -0.17) was determined. In addition, there was a significant negative correlation between BMD values at lumbar spine (L1-L4) and right and left femoral neck with s-NTx (r = -0.26). Both BMD values at right and left femoral neck were showed a significant positive correlation with s-Ca (r = 0.11), and s-PINP (r = 0.16), while a significant negative correlation with s-NTx (r = -0.23). Serum vitamin B12 was showed a significant negative correlation with s-homocysteine (r = -0.19), CTx (r = -0.15), and NTx (r = -0.12). Serum folate was showed a significant positive correlation with BMD values at right and left femoral neck (r = 0.17) and s-Ca (r = 0.17). Finally, serum homocysteine was showed a significant negative correlation with BMD values at lumbar spine (L1-L4) (r = -0.16) and right and left femoral neck (r = -0.15).

Table 3 represent a comparison between MTHFR genotypes; homozygous (TT) (n=8), heterozygous (CT) (62), and wild type (CC) (n=245) by using ANOVA. A significant differences were found in s-Ca levels in wild (2.41±0.12.), heterozygous (2.36±0.10), and homozygous (2.51±0.19). Also there were a significant differences in s-homocysteine levels in wild (11.44±2.80), heterozygous (12.31±2.74), and homozygous (20.41±1.54) (P<0.05). Finally, s-OC levels showed a significant differences in all three groups with higher level in wild type and lower level in homozygous.

4. Discussion:

Both men and women lose bone at relatively slow rates starting at age 40, with women more rapidly than men with menopause onset in their late 40 years or early 50 years **(Bart *et al.*, 2010)**. Our subjects are normal healthy men, their ages ranged 20-50 years, but the prevalence of osteopenia and osteoporosis is high if we but in our mind the small age of our subjects. The prevalence of osteopenia was 22.8%, while osteoporosis 2.5%. **Mir Sadat and AlElq (2011)** study among Saudi men aged from 60 to 76 years attending outpatient orthopedic and internal medicine clinic found that, the prevalence of osteopenia was about 64%, while osteoporosis was 33.9%. The higher prevalence of osteoporosis and osteopenia compared with our study regarding to two concept. The first one is a high range of age in their subjects which the smaller one aged 60 years, while smaller one in our subject was 25 years old. The second concept was, their subject may suffering from any diseases because they were attending clinic due to some reason, but our subject were healthy. Age remained an important predictor of hip fracture even after adjusting BMD **(Bart *et al.*, 2010)**. In our study, a significant difference in age with smaller age in normal and higher in osteoporotic group was found. In addition, a highly significant negative correlation between age and BMD at right and left femoral neck was present in our entire groups. This in agreement with previous study of **Bagher *et al.* (2005)**, they found a significant negative association between BMD and age in Iranian men. Our results also showed a high significant negative correlation of age with s-Ca and bone turnover markers (OC, PINP, NTx, and CTx), and these add another support for increase bone loss in elderly.

Table 1: Comparison of age, BMI, BMD at lumbar spine (L1-L4) and right and left femoral necks, s-Ca, s-folate, s-homocysteine, s-OC, s-PINP, s-CTx, and s-NTx in normal, osteopenic and osteoporotic individual (ANOVA):

Variables	**Normal (n = 235)**	**Osteopenic (n = 72)**	**Osteoporotic (n = 8)**	***P* value**
Age (years) (mean±SD) (range)	34.84±9.33 20-50	36.96±10.07 20-50	41.64±7.43 34-50	* 0.030
BMI (mean±SD) (range)	28.44±4.72 16.50- 38.40	26.64±4.71 17.40-38.30	25.05±3.50 21.10-30.30	* 0.002
BMD (L.S) (g/cm) (mean±SD) (range)	1151.6±108.7 913-1528	961.79±78.66 829-1256	869.36±87.48 747-973	* 0.000
BMD (R.F.N) (g/cm) (mean±SD) (range)	1044.7±106.7 680-1434	872.53±93.62 695-1108	759.45±80.13 624-889	* 0.000
BMD (L.F.N) (g/cm) (mean±SD) (range)	1049.7±106.0 860-1434	868.77±94.88 694-1091	726.82±77.78 640-873	* 0.000
s-Ca (mmol/L) (mean±SD) (range)	2.40±0.10 2.17-2.74	2.39±0.11 2.14-2.75	2.33±0.69 2.21-2.43	0.074
s-Vitamin B12 (pg/ml) (mean±SD) (range)	338.8±106.4 129-594	215.15±84.03 158-466	250.18±19.22 222-278	* 0.016
s-Folate (ng/ml) (mean±SD) (range)	12.29±3.85 3.4-14.7	7.59±1.49 2.88-10.26	6.6±4.64 3.32-9.88	* 0.007
Homocysteine(mmol/L)(mean±SD) (range)	10.97±3.19 3.2-17.7	12.9±3.12 7.50-26.20	13.97±4.02 10.10-23.02	* 0.005
s-OC (mmol/L) (mean±SD) (range)	27.05±11.74 9.80-69.64	27.99±11.52 9.80-64.61	23.37±6.40 13.90-32.10	0.340
s-PINP (mmol/L) (mean±SD) (range)	68.50±37.06 14.35-207.8	57.12±21.93 13.25-105.30	46.78±10.44 32.01-64.23	0.188
s-CTx (mmol/L) (mean±SD) (range)	508.15±195.6 110-984	603.88±275.6 211-1231	674.45±143.36 550-950	* 0.049
s-NTx (mmol/L) (mean±SD) (range)	741.51±267.2 57-1417	1177.80±717.3 257-3311	1290.50±200.48 1043-1799	* 0.000

BMI(body mass index), BMD (bone mass density), L.S(lumbar spine), R.F.N (right femoral neck), L.F.N (left femoral neck), Ca (calcium), OC (osteocalcin), PINP (pro-collagen type I amino propeptide), CTx (carboxy-terminal telopeptide), NTx (amino-terminal telopeptide). * $P<0.05$ is considered significant.

Table 2: Correlation among age, BMI, BMD at lumbar spine (L1-L4), and right and left femoral neck, s-Ca, s-vitamin-B12, s-Folate, s-homocysteine, s-OC, s-PINP, s-CTx, and s-NTx in all individuals by using Pearson`s correlation.

Variable	Age	MI	BMD1	BMD2	BMD3	Ca	B12	Folate	Homo	OC	PINP	CTx	NTx
Age		** 0.38		** -0.27	** -0.27	** -0.26				** -0.48	** -0.55	** -0.44	** -0.17
BMI			** 0.20	** 0.15	** 0.15	** -0.19				** -0.33	** -0.33	** -0.27	** -0.17
BMD1				** 0.62	** 0.62				** -0.16				** -0.26
BMD2					** 0.96	* 0.11		** 0.17	** -0.15		** 0.16		** -0.23
BMD3						* 0.12		** 0.17	** -0.15		** 0.16		** -0.23
Ca								** 0.17	* 0.13				* -0.13
B12									** -0.19			** -0.15	* -0.12
Folate													
Homo													
OC											** 0.79	** 0.54	** 0.26
PINP												** 0.57	** 0.21
CTx													** 0.37

BMI(body mass index), BMD1 (bone mass density at lumbar spine), BMD2 (bone mass density at right femoral neck), BMD2 (bone mass density at left femoral neck), Ca (calcium), vit-B12 (vitamin B12), Homo (homocysteine) OC (osteocalcin), PINP (pro-collagen type I amino propeptide), CTx (carboxy-terminal telopeptide), NTx (amino-terminal telopeptide).

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

Table 3: Comparison of age, BMI, BMD at lumbar spine (L1-L4) and right and left femoral necks, s-Ca, s-vitamin B12, s-folate, s-homocysteine, s-OC, s-PINP, s-CTx, and s-NTx in wild (C-C), heterozygous (C-T), and homozygous (T-T) by using (ANOVA):

Variables	**Wild (C-C) n = 245**	**Heterozygous (C-T) n = 62**	**Homozygous (T-T) n = 8**	***P* value**
Age (years) (mean±SD) (range)	35.2±9.4 20-50	36.2±10.0 20-50	34.8±10.5 22-49	0.700
BMI (mean±SD) (range)	27.81±4.77 15.80-38.40	28.14±4.97 17.40-38.30	28.83±5.15 22.80-38.40	0.660
BMD (L.S) (g/cm) (mean±SD) (range)	1110.8±108.7 797-1528	1080.6±141.30 799-1394	1073.7±64.22 747-973	0.299
BMD (R.F.N) (g/cm) (mean±SD) (range)	996.33±129.8 675-1434	1007.6±137.1 691-1330	1008.2±114.4 804-1093	0.835
BMD (L.F.N) (g/cm) (mean±SD) (range)	995.18±133.5 659-1409	1016.5±147.0 640-1362	1008.2±114.1 804-1093	0.534
s-Ca (mmol/L) (mean±SD) (range)	2.41±0.12 2.20-2.97	2.36±0.10 2.14-2.72	2.51±0.19 2.33-2.82	* 0.001
s-Vitamin B12 (pg/ml) (mean±SD) (range)	360.99±148.1 158-959	359.00±134.87 158-771	433.67±225.0 129-736	0.572
s-Folate (ng/ml) (mean±SD) (range)	9.49±3.09 3.32-19.82	8.51±2.37 2.88-13.11	8.92±3.07 5.82-14.28	0.064
Homocysteine(mmol/L)(mean±SD) (range)	11.44±2.80 4.60-21.40	12.31±2.74 7.20-19.90	20.41±1.54 7.50-44.00	* 0.000
s-OC (mmol/L) (mean±SD) (range)	28.11±12.24 9.80-75.01	25.58±10.87 11.58-64.61	21.23±10.19 11.30-38.12	* 0.038
s-PINP (mmol/L) (mean±SD) (range)	66.93±35.51 13.25-207.8	65.53±36.41 20.46-153.3	42.23±14.97 15.19-60.14	0.130
s-CTx (mmol/L) (mean±SD) (range)	573.82±266.3 110-1320	545.77±241.6 143-1400	507.17±241.6 210-911	0.646
s-NTx (mmol/L) (mean±SD) (range)	1043.0±675.8 87-3116	946.7±677.0 28-2844	785.5±272.6 484-1259	0.389

BMI(body mass index), BMD (bone mass density), L.S (lumbar spine), R.F.N (right femoral neck), L.F.N (left femoral neck), Ca (calcium), OC (osteocalcin), PINP (pro-collagen type I amino propeptide), CTx (carboxy-terminal telopeptide), NTx (amino-terminal telopeptide).
* $P<0.05$ is considered significant

Various studies have shown a positive correlation between BMD and BMI. **Dogan *et al.* (2010)** found a positive correlation between BMI and BMD among Turkish men. Also **El-maghraoui *et al.* (2009)** found a positive association between BMI and BMD at lumbar spine (L1-L4) and right and left femoral neck in Moroccan men. In our study, a significant differences in BMI between normal, osteopenic, and osteoportic was present, with higher value in normal individuals. Moreover, a highly significant positive correlation between BMI and BMD at lumbar spine (L1-L4) and right and left femoral neck was showed in our entire group.

Vitamin B12 and folate play important role in homocysteine metabolism and reduce its blood level. hyperhomocysteinemia is a common age related problem in elderly people **(Clarke *et al.*, 2003)**. The main reasons for hyperhomocysteinemia in elderly people are deficiencies of folate and vitamin B12. Several studies have focused on whether high plasma homocysteine concentrations and a low folate and vitamin B12 status are associated with a decreased BMD and an increased fracture risk or not. All prospective epidemiologic trials recording more than 1000 patients found a significant positive relation between homocysteine plasma levels and fracture risk **(McLean *et al.*, 2004)**. Some studies suggest that the relation between homocysteine and fractures is more pronounced in men than in women **(Dhonukshe-Rutten *et al.*, 2003; Brustolin *et al.*, 2010)**. The high circulating homocysteine concentrations can easily be modified by a simple and safe supplementation of folate and vitamin B12 **(Clarke *et al.*, 2003)**. Folic acid fortification is not mandatory in some countries as USA, and it is only applied on small scale in bread substitutes but not in our country. Because that, our study is an excellent opportunity to investigate the relation of folate and vitamin B12 level and BMD in non-fortified population. **Robert *et al.* (2008)** study among old men and women showed that, lower concentrations of vitamins B12 and folate were associated with increased risk of hip fracture, and they conclude, lower plasma vitamin B12 and folate concentration may, to some degree, explain the

association between elevated plasma homocysteine and increased hip fracture risk. Vitamin B12 deficiency was recorded in about 19% of our subjects, and our study showed a significant differences in vitamin B12 levels among normal, osteopenic, and osteoporotic groups, with higher level associated with normal individuals. In addition, a highly significant negative correlation between vitamin B12 and homocysteine levels was present among entire groups. As previous studied done by **Clara *et al.* (2006)**, we did not find a correlation between vitamin B12 levels and BMD of lumbar spine and right and left femoral neck. More than vitamin B12 deficiency, the folate deficiency was recorded in 26% of our subject. In accordance with **Robert *et al.* (2008)**, a significant differences in serum folate levels was found among the three groups, with higher level in normal individuals, while lower level associated with osteoporotic patients. In addition a highly significant positive correlation between folate level and BMD at right and left femoral neck and s-Ca levels were found among entire groups. Fifteen percent of our subjects were suffered from mild hyperhomocysteinemia. In relation with homocysteine levels, our results in accordance with **Robert *et al.* (2008); and Zora *et al.* (2009)** studies. They found an association between elevated homocysteine level and low BMD values at right and left femoral neck. A significant differences in homocysteine levels were found among entire groups,with higher level in osteoporotic group. A highly significant negative correlation between homocysteine and BMD at lumbar spine and right and left femoral neck in the entire groups was found. In addition a significant positive correlation between homocysteine and s-Ca level among entire groups was present, and these may due to increase resorption activity.

The methylenetetrahydrofolate reductase (MTHFR) gene is located on chromosome 1 at 1p36.3. MTHFR is a key enzyme in folate metabolism. Its deficiency usually leads to a significant reduction in plasma concentrations of folate, vitamin B12 and methionine, whereas homocysteine is increased **(Erdogan *et al.*, 2010)**. A number of studies have investigated the role of the C677T MTHFR polymorphism on bone phenotypes in older men and women with conflicting results. **Abrahamsen *et al.* (2003)** and **Miyao *et al.*, (2000)** studies found significant genotype effects for the lumbar spine and hip, but results for total body BMD were inconsistent. These findings applied to women only. Our study in accordance with **Colin *et al.* (2009)**, that show a significant elevated level of homocysteine with homozygote and heterozygote compared with wild one, but there was no significant differences in BMD for three types, and this may due to the small number of subjects in homozygote groups.

Recomindation and conclusion: We conclude that, vitamin B12, folate, and MTHFR C677T polymorphism may affect BMD in Saudi men indirectly through its effect on homocysteine levels. So, Supplementation or changes in diet are easy and effective methods for controlling vitamin B12 and folate status, and control homocysteine in normal level, and may be considered as potential novel measures for reducing fracture rates. Further research is needed to determine how homocysteine and B vitamins influence fracture risk, and whether these interventions may help maintain bone mass and reduce the risk of fracture in elderly men and women

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