Measurement of Total Plasma Homocysteine, Folic Acid, Vitamin B12 and Vitamin D3 Levels among Osteoporotic Women in Duhok City

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ARTICLE INFO
Submission date: 29 /7 2019
Acceptance date: 2 / 9/ 2019
Publication date: 31 /12 /2019

Abstract

Background and objectives: Recent studies suggest that high homocysteine levels are associated with an increased risk of fractures. Homocysteine levels are known to be influenced by vitamin B_{12}, vitamin D_3 and folic acid status. Elevated plasma total homocysteine (tHcy) and deficiencies of folate and vitamin B12 are associated with risk of osteoporosis and bone loss. Therefore, this study aims to examine whether high plasma levels of homocysteine and low plasma levels of folate, vitamin B_{12} and vitamin D_3 predicted a bad prognosis of bone health or not.

Methods: A cross sectional study was conducted on (90) women whose ages were between (45–85 years old) in 2017–2018. Recorded information on T – score values were obtained from computerized DEXA machine at the Duhok Rheumatic disease and Rehabilitation center. Independent t – test by (SPSS version 23) program was used to estimate relationship and risk assessment among study parameters comparing to levels of plasma total homocysteine (tHcy).

Results: Hyperhomocysteinemia is associated with skeletal abnormalities and osteoporosis. We tested whether levels of homocysteine and critical co-enzymes of homocysteine metabolism, such as vitamin B_{12}, D_3 and folate which are related to bone mineral density (BMD) measured by DEXA. The current data demonstrates that homocysteine is highly significant (*P* value < 0.01) with each of age, waist circumferences (C. W.), vitamin B_{12}, folic acid, vitamin D_3 and T- score quartiles, also significant (*P* value < 0.05) with Body Mass Index (BMI), calcium (Ca^+) and Total Body Fat percent (TBF %).

Conclusions: Homocysteine seems to be a predictor for development of osteoporosis among study population (elderly menopaused women in Duhok city). The present data suggest a major association between folate, vitamin B_{12}, vitamin D_3 and bone mineralization. Our data documented the hypothesis that homocysteine may play a role in the pathogenesis of osteoporotic fractures.

Key Words: Osteoporosis, Hyperhomocysteinemia, fracture, postmenopausal women.

Introduction

Osteoporosis is an endless and multifactorial confusion that described via low bone mass and microarchitectural weakening of bones [1]. It has been recorded that the significant outcome of osteoporosis is bone fracture. Particularly hip fractures are as often as possible related with standardization and expanded mortality, and in this manner with an expanded social and monetary weight [2]. Deficiency of vitamin B12 and folic acid status as well as elevation of homocysteine concentration (more than 15μmol/L) has been related in a few investigations with lower bone mineral density which resulted in increasing the incidence of bone fracture in elderly women [3]. The reason is due to a combination of natural and hereditary components, nutrition, lifestyle, and hormonal elements [4]. Vitamin B12 and folic acid are significant elements of homocysteine metabolism and regulating its plasma concentrations [5]. Hypothetically, switching raised concentrations of homocysteine via supplementation of folate and vitamin B12 could preserve the issue of hindered bone tissues healthiness [6].

Calcium and vitamin D3 play an important role in preservation and healthiness of bone tissues. In addition, it has been stated that around 50% of women that are under the treatment of osteoporosis have low level of Vitamin D3 as well as about 90% of women may not take sufficient amount of calcium. Recent studies have found out that recovery from osteoporotic disease is improved by a number of medications which lead to minimize the risk of bone fractures. The presence of adequate amount of Ca and Vitamin D3 in these medications will achieve this improvement [7].

Osteoporosis in menopaused women is mostly caused by estrogen inadequacy, while, natural and lifestyle factors as well as metabolic and hereditary clutters are contributing [8]. The World Health Organization has characterized osteoporosis as a Bone Mineral Density (BMD) which accounts more than 2.5 standard deviations (SD) beneath the mean for typical youthful women [8].

To summarize, osteoporosis is the most widely recognized type of the malady, influencing a large portion of the skeleton [9]. There are a wide range of kinds of osteoporosis. The most widely recognized type of osteoporosis is known as "essential osteoporosis", osteoporosis that is not caused by some other particular issue. Bone misfortune is caused by particular sicknesses or meds is alluded to as "optional osteoporosis." [10].

Materials and Methods

Study design and study period

The present cross-sectional study was conducted at the Duhok Rheumatic Disease and Rehabilitation Center, during the period of November 2017 until February 2018.

The population enrolled in this Study

The samples were collected randomly, previously prepared questioner was designed according to inclusion criteria, only (90) samples were selected from patients and asked to participate in this study project. The evaluation of osteoporosis level after taking Dual-energy X-ray absorptiometry (DEXA) which is the gold standard method for diagnosis of osteoporosis in Rheumatoid Center in Duhok city. The respondents (osteoporotic women) were grouped into eight different classes on the basis of age, Waist circumferences (WC),

Body mass index (BMI), plasma levels of Vitamin D3, vitamin B12, Folic Acid, Homocysteine and quartiles of T-Score.

Inclusion and Exclusion criteria:

The inclusion criteria for the current study was as follow:
Menopaused women aged 40 and above, Osteoporotic women with T – Score -2.5 and more and Patients not on study parameters medications particularly multivitamins containing vitamin B12, vitamin D3 and folic acid. While the Exclusion criteria for the present study was as follow:

- Normal and osteopenic women.
- Receiving of hormone replacement therapy.
- Patients suffering from renal diseases.
- Patients suffering from liver disorders.
- Patients under the effect of chemotherapy.
- Patients with type I and type II diabetes mellitus.
- Patients on medication particularly multivitamins and antioxidants.

Biochemical Analysis

Measurement of Plasma total homocysteine (tHcy).

Detection of Plasma total homocysteine (tHcy), folic acid, vitamin B12, vitamin D3 and calcium using specific kits (Roche) according to company protocol instructions using Cobass 6000 autoanalyzer machine [11].

Test principle

Homocysteine Enzymatic Assay is based on a novel enzyme cycling assay principle that assesses the co‑substrate conversion product instead of assessing co‑substrate or Hcy conversion products of Hcy, In this assay, oxidized Hcy is first reduced to free Hcy which then reacts with a co‑substrate, S‑adenosylmethionine (SAM), to form methionine (Met) and S‑adenosylhomocysteine (SAH), catalyzed by a Hcy S‑methyltransferase. SAH is assessed by coupled enzyme reactions where SAH is hydrolyzed into adenosine (Ado) and Hcy by SAH hydrolase, and Hcy is cycled into the Hcy conversion reaction to form a reaction cycle that amplifies the detection signal. The formed Ado is immediately hydrolyzed into inosine and ammonia. In the last step, the enzyme glutamate dehydrogenase (GLDH) catalyzes the reaction of ammonia with 2‑oxoglutarate and NADH to form NAD^+. The concentration of Hcy in the sample is directly proportional to the amount of NADH converted to NAD^+ ($\Delta A_{340\,nm}$) [11].

Assay:

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1/R2-S-SR
Reaction direction	Decrease
Wavelength A/B	340/659 nm
Calc. first/last	50/62
Unit	µmol/L

Time required

Each run we put 10 samples / 30 minutes.

Statistical analysis

Data was analyzed by using the Statistical Package for Social Sciences program (SPSS) version (23). T - test was used to compare between proportions. A P value of ≤ 0.05 was considered statistically significant, while P value was 0.01 it considered statistically as a highly significant. One Way ANOVA was used to compare among more than two groups. The Pearson correlation was used to determine the correlation coefficient between Homocysteine and study parameters.

Results

Demographic and laboratory characteristics of the study population are included in Table 1.

Table (1) Frequency distribution of the study variables.

Study variables	N	%
Age (years)		
40-50	18	20
51-60	26	29
61-70	32	36
>70	14	15
BMI (kg/m2)		
Normal	9	9.10
Overweight	25	25.28
Obese	56	56.62
WC (cm)		
Normal	17	88
Abnormal	73	12
T-Score		
(-2.5)-(-3.49)	38	45
(-3.5)-(-4.49)	21	21
(-4.5)-(-5)	31	34
Homocysteine (µmol/L)		
Normal	15	14
Abnormal	75	86
Folic acid (ng/ml)		
Low	21	23
Normal	69	77
Vitamin B12 (pg/ml)		
Low	26	29
Normal	64	71
Vitamin D3 (ng/mL)		
Deficient	13	15
Insufficient	63	69
Sufficient	14	16

Homocysteine and study parameters

It can be seen from table 2 that there was a definitely significant relationship between homocysteine and all study parameters.

Table 2 Comparison of Demographics and Biochemical measurements in Normal Homocysteine & High Homocysteine Osteoporotic Women.

Study variables	Mean & SD of Osteoporotic Women with Normal Homocysteine (n = 15)	Mean & SD of Osteoporotic Women with High Homocysteine (n = 75)	P-value
Age	49.86 ± 8.16	61.82 ± 9.09	< 0.01
Waist circumferences (cm)	98.13 ± 12.41	108.45 ± 14.22	< 0.01
Body mass index (Kg/m^2)	31.03 ± 4.98	33.04 ± 5.85	< 0.05
Vitamin D3 (ng / ml)	25.71 ± 6.82	19.09 ± 8.66	< 0.05
Vitamin B12 (pg / ml)	663.87 ± 151.83	312.06 ± 152.21	< 0.01
Folic acid (ng / ml)	12.27 ± 3.72	5.26 ± 3.76	< 0.01
T – Score	-2.84 ± .56	-3.96 ± .73	< 0.01
Total Body Fat (TBF) (%)	40.52 ± 8.79	43.44 ± 8.92	< 0.05

Vitamin B12 and study variables

Regarding vitamin B12, table 3 shows that there was a highly significant differences in vitamin B12, age, vitamin D3, homocysteine and T – score ($P < 0.01$).

Table 3 Comparison of Demographics and Biochemical measurements in Normal Vitamin B12 & Low Vitamin B12 of Osteoporotic Women

Study variable	Mean & SD of Osteoporotic Women with Normal Vitamin B12 (n = 64)	Mean & SD of Osteoporotic Women with Low Vitamin B12 (n = 26)	P-value
Age	55.78 ± 7.85	69.80 ± 7.26	< 0.01
Waist circumferences (cm)	105.32 ± 13.80	110.19 ± 15.51	NS
Body mass index (Kg/m^2)	32.42 ± 5.34	33.41 ± 6.66	NS
Vitamin D3 (ng / ml)	22.13 ± 7.35	15.42 ± 10.03	< 0.01
Folic acid (ng / ml)	8.29 ± 4.14	1.85 ± .46	< 0.01
Homocysteine (µ mol /L)	17.24 ± 2.64	24.18 ± 2.54	< 0.01
T – Score	-3.44 ± .72	-4.59 ± .24	< 0.01
Total Body Fat (TBF) (%)	42.54 ± 8.71	43.97 ± 9.50	NS

Folic acid and study variables

Regarding folic acid, there was a highly significant relation in age, vitamin D3, homocysteine vitamin B12, T – score and waist circumferences ($P < 0.01$).

Table 4 demonstrate the Comparison of Demographics and Biochemical measurements in normal folic acid and low folic acid of osteoporotic women.

Study variables	Mean & SD of Osteoporotic Women with Normal Folic acid (n = 69)	Mean & SD of Osteoporotic Women with Low Folic acid (n = 21)	P-value
Age	56.95 ± 8.78	69.28 ± 7.56	< 0.01
Waist circumferences (cm)	104.56 ± 13.77	113.85 ± 14.43	< 0.05
Body mass index (Kg/m^2)	32.08 ± 5.20	34.76 ± 6.98	NS
Vitamin D3 (ng / ml)	23.60 ± 11.88	13.37 ± 8.45	< 0.01
Vitamin B12 (ng / ml)	431.29 ± 191.33	171.57 ± 21.12	< 0.01
Homocysteine (µ mol /L)	17.51 ± 2.72	24.93 ± 2.24	< 0.01
T – Score	-3.51 ± .75	-4.63 ± .18	< 0.01
Total Body Fat (TBF) (%)	42.31 ± 8.79	45.06 ± 9.20	NS

Vitamin D3 and study parameters

Regarding vitamin D3 as shown in table 5, there was a highly significant differences among sufficient and insufficient vitamin D3 with each of waist circumference, body mass index and total body fat. In addition, as shown in table 5, it can be seen that there was a highly significant difference among sufficient and deficient vitamin D3 and all study parameters except serum calcium. Finally, taking all together, there was a highly significant relationship between vitamin D3 and all study parameters except serum calcium, vitamin B12 and folic acid.

Table 5: One-way ANOVA analysis comparison of demographics and biochemical measurements in sufficient vitamin D3, insufficient vitamin D3 and deficient vitamin D3 of osteoporotic women.

Study variables	Mean & SD of Osteoporotic Women with Sufficient Vitamin D3 (n = 14)	Mean & SD of Osteoporotic Women with Insufficient Vitamin D3 (n = 63)	Mean & SD of Osteoporotic Women with Deficient Vitamin D3 (n = 13)	P-value
Age	58.07 ± 13.36	57.85 ± 8.19	70.35 ± 6.72	< 0.01
Waist circumferences	93.14 ± 7.67	109.64 ± 12.71	107.42 ± 19.04	< 0.01
Body mass index (Kg/m^2)	27.17 ± 3.15	33.94 ± 5.48	32.76 ± 5.84	< 0.01
Vitamin B12 (ng / ml)	440.58 ± 224.97	388.10 ± 198.86	223.69 ± 97.77	NS
Folic acid (ng / ml)	8.43 ± 4.69	6.68 ± 4.60	3.31 ± 2.52	NS
Homocysteine (µ mol /L)	17.20 ± 3.92	18.83 ± 3.64	23.10 ± 3.99	< 0.01
T – Score	-3.42 ± .90	-3.69 ± .76	-4.48 ± .54	< 0.01
Total Body Fat (TBF) (%)	32.57 ± 6.27	45.17 ± 8.11	43.53 ± 7.50	< 0.01

Correlation analysis:

According to Pearson correlation coefficient (r). Homocysteine (µ mol /L) in study sample was highly significant correlations between all study variables including age in years, body mass index (Kg/m^2), waist circumferences (cm), vitamin D3 (ng / ml), Folic acid (ng / ml), vitamin B12 (pg / ml), homocysteine (µ mol /L), T - Score and Total Body Fat (TBF %), table (6).

Table 6: Correlation between homocysteine (µ mol /L) and other related parameters in osteoporotic women.

Variables	(r)	P-value
Age	.745	< 0.01
Body mass index (Kg/m^2)	.281	< 0.01
Waist circumferences (cm)	.376	< 0.01
Vitamin B12 (pg / ml)	-.832	< 0.01
Folic acid (ng / ml)	-.851	< 0.01
Vitamin D3 (ng / ml)	-.557	< 0.01
T – Score	-.847	< 0.01
Total Body Fat (TBF) (%)	.255	< 0.05

Folic acid (ng / ml) in osteoporotic women was highly significant correlations between age in years, waist circumferences (cm), vitamin D3 (ng / ml), vitamin B12 (pg / ml), homocysteine (µ mol /L) and T – Score table (7) and there was significant correlation

with body mass index (Kg/m^2). But folic acid (ng / ml) in osteoporotic women group was non-significant only Total Body Fat (TBF %).

Table 7: Correlation between folic acid (ng / ml) and other related parameters in study patients.

Variables	(r)	P-value
Age	-.600	< 0.01
Body mass index (Kg/m^2)	-.264	< 0.05
Waist circumferences (cm)	-.363	< 0.01
Vitamin B12 (pg / ml)	.775	< 0.01
Vitamin D3 (ng / ml)	.433	< 0.01
Homocysteine (µ mol /L)	-.851	< 0.01
T – Score	.811	< 0.01
Total Body Fat (TBF) (%)	-.204	NS

Vitamin B12 (pg / ml) in osteoporotic women was highly significant correlations between age, waist circumferences (cm), vitamin D3 (ng / ml), folic acid (ng / ml), homocysteine (µ mol /L) and T - Score table (8). However, vitamin B12 (pg / ml) in osteoporotic women group was non-significant with body mass index (Kg/m^2) and Total Body Fat (TBF %).

Table 8: Correlation between vitamin B12 (pg / ml) and other related parameters in osteoporotic women.

Variables	(r)	P-value
Age	-.693	< 0.01
Body mass index (Kg/m^2)	-.183	NS
Waist circumferences (cm)	-.342	< 0.01
Folic acid (ng / ml)	.775	< 0.01
Vitamin D3 (ng / ml)	.461	< 0.01
Homocysteine (µ mol /L)	-.832	< 0.01
T – Score	.816	< 0.01
Total Body Fat (TBF) (%)	-.182	NS

Discussions

Overview

Recent epidemiological researches demonstrated that bone diseases and bone losses closely associated with prominent elevation of Homocysteine level in blood known as Hyperhomocysteinemia [12]. This increase in homocysteine concentration resulted in disruption in its metabolism, however, homocysteine metabolism is mainly controlled by folate and vitamin B12 [13]. furthermore, when there is defect in the action of methylenetetrahydrofolate (MTHFR) reductase enzyme. In fasting conditions Mild

hyperhomocysteinemia might be observed due to the mild impairment in the methylation pathway (i.e. inadequate amount of each of vitamin B12 and folic acid or (MTHFR) defect) [14].

Studies have demonstrated that Hyperhomocysteinemia (> 15 mmol/L) raise the activity of osteoclast and remodeling of bones. Furthermore, this will lead to an increase the impact of homocysteine on development of bones [15]. Kang and Trelstad (1972) discovered that homocysteine interfered with collagen cross-linking from filtered rodent skin collagen, However, high concentration of homocysteine represses the action of lysyl oxidase (a catalyst associated with cross- linking of collagen) and in this manner animate osteoclast action in lifted fixation.

The present study demonstrates that high levels of serum Homocysteine were positively correlate and highly significant (*P* value < 0.01) with plasma vitamin B12, vitamin D3 and folic acid concentrations. This clarify that in the present study plasma homocysteine might have a direct impact in the pathogenesis of osteoporosis and other related bone diseases which lead to pathological fractures in elderly persons and might be considered as a predisposing factor for bone loss.

The data from present study shows that plasma level of homocysteine is closely related to plasma levels of vitamin B12, vitamin D3 and folic acid. Furthermore, the current data clarifies that Homocysteine is highly significant (*P* value < 0.01) with each of age, waist circumferences (CW), vitamin B12, folic acid, vitamin D3 and T- score quartiles and significant (*P* value < 0.05) with BMI (Body Mass Index) and total body fat percent (TBF %) on the other hand, Homocysteine were also positively corelated with each of age, BMI, TBF, WC, T – score quartiles.

Plasma Homocysteine and Age

The ages of menopaused women who have participated in the current study whose ages were between (40 – 85 years old). Homocysteine was positively correlated with age (table 3.6) meaning that the homocysteine level increased by the age. The Hyperhomocysteinemia in elderly women will affect on bone mineralization and remodeling by elimination of calcium and minerals from the bone as well as bone fragility and then fracturing [16]. Aging progress will decrease body response to many nutrient factors, which might have a great role to keep body minerals in balance, for instance, decrease in the activation of vitamin D3 in kidneys in elderly (after exposing the subcutaneous fat to the sun light, final activation of vitamin D3 will take place in kidneys) due to elderly age as a result of decreased renal response to parathyroid hormone and inadequate absorption of calcium from the intestinal tract. However, elderly women will need sufficient amount of PTH to stimulate vitamin D3 activation and production. Cholecalciferol play an essential role in the pathogenesis of osteoporosis, so it's decrease will have a negative effect on bone texture and development of senile osteoporosis. A Systematic Review with Meta-Analyses done by van Wijngaarden J. P. et al 2012 shows a strong relationship between bone loss (osteoporosis) T – score > -2.5 and age. Similar results were recorded in the current study as shown in tables (3.14 and 3.15).

Plasma Homocysteine and Obesity Parameters

Previously, it has been stated that diseases of bones (bone loss) and obesity are two irrelevant illnesses, nowadays there are several researches demonstrated that obesity and bone diseases are closely related to each other and both share many predisposing factors [17]. For instance, environmental and genetic factors. Body weight composed of body fluids, soft tissues and hard tissues, fat (which is a component of soft tissues) is regarded as the main component of body weight, after maturity only body fat is changeable (either by exercise or by food regimen). Recent studies demonstrated that total body fat which is the most prominent guides of obesity, may have a beneficial effect on bone health. The assimilation and differentiation of osteoblast and adipocyte is created as a result of the maturation of common precursor stem cells. Furthermore, production of hormones which have a direct and great effect on bone health known as adipocyte – derived hormones [18].

- **Waist circumferences**

Taking waist circumferences as a marker for obesity in patients with osteoporosis and studying serum homocysteine in those patients has been conducted by several studies. All of them concluded that W. C. was closely correlated with bone loss [19]. Similarly, our data also demonstrated that hyperhomocysteinemia was significantly seen in patients with high W. C. Al- Bayyari N. et al. (2017) conclude that in overweight women elevation of plasma homocysteine concentration is prominently seen in women with abnormal WC measurement, and it might be regarded as independent predictor of plasma homocysteine level [19].

- **Body mass index (BMI)**

In present study, the obese and overweight patients (women) with osteoporosis whose T – score was (> -2.5), were documented by DEXA machine had high values of plasma homocysteine (> 15 mmol / L) could be seen. Study conducted by Carranza-Lira S. et al. (2002) and Asomaning K. et al. (2006) Summarized that overweight and obese women with high BMI (> 25 Kg / m^2) are at increased risk of osteoporosis [20].

- **Total Body Fat (TBF %):**

Similarly, W.C. and BMI the TBF also were positively corelated with homocysteine concentration. This concludes that the current study concluded that the Obesity parameters (WC, BMI and TBF) have effect on homocysteine level in positive manner as shown in (Table 3.6). Several studies concluded that adipose tissue (body fat) is not functionless body constituent, furthermore, it is responsible for production and secretion of several biologically active substances, for example, resistin, estrogen hormone, adiponectin and interleukin – 6 (IL-6). These substances either directly or indirectly play an important and effective role as a source of energy, also play a great role in bone health and body metabolisms. On the other hand, many endocrine organs also have a great role in bone health, for instance, the pancreas which produce molecules called bone – active substances such as amylin and insulin. The action and effect of such molecules throughout the body including bone may interpret the relationship between bones and body fat [21]. Finally, osteoblast and adipocyte originate from the same source of cells known pluripotential mesenchymal stem cells. Normally, this stem cells equally differentiated into both osteoblasts and adipocytes, however, this differentiation is under

control and regulated by many interacting mechanisms and pathways which may participate to the final effect of body fat on body bones. Some enzymes indirectly play a role in bone homeostasis, for example, aromatase enzyme, which presents in adipose tissue and gonads. This enzyme is responsible for the synthesis of estrogen hormone by using testosterone or androstene, as well as estrogen have a great role in bone health and protect bones against osteoporosis by make an inhibition to osteoclast action and bone resorption, and at the same time make a stimulation to osteoblast cells which have a great role in bone remodeling. In this case the elderly women (postmenopausal women) are lack to estrogen hormone as a result of the absence of menstrual cycle as well as the ovaries no longer produces estrogen, so extragonadal estrogen which is mainly produced from adipose tissue and become the main source of estrogen and may compensate the stopped estrogen from the workout ovaries [22].

Therefore, in obese postmenopausal women a high amount of body fat suggested to have a protective role on bones because of elevation in production of extragonadal estrogen which will have a potential effect on preserving bones from losses. In postmenopausal women (elderly women) any decrease in estrogen level will cause an increase in central body fat as well as increase chance of bone turnover and then bone loss will be accelerated. Furthermore, recent studies show that declining in endogenous estrogen level in elderly women complimented by declining in osteoblasts numbers and elevation in adipocyte counts. For that reason, in postmenopausal women estrogen replacement therapies will prevent or reduces menopause – induced fat gains and decreases the occurrence early postmenopausal osteoporosis. To sum up, the incompatible relationship between total body fat percent (TBF %), body mass index (BMI) and total plasma homocysteine (tHcy) have been reported in the general population. The present research reviewed the hypothesis that increased plasma level of homocysteine are closely associated with increased W.C. levels, overweight and obese women with BMI (25 – 29.9 Kg / m2) (more than 30 Kg / m2) respectively and total body fat percent elevation. In Duhok City, obesity and overweight are prevalent among elderly women and hyperhomocysteinemia, along with obesity and overweight, are independent risk factors for osteoporosis.

Plasma Homocysteine and Nutritional factors

Some reports illustrate the relationship between raised plasma homocysteine and fracture threats, while others discover no relationship. Nonetheless, it is not an evident whether this is identified with aggregate homocysteine or not, or the measurement of vitamin D3, vitamin B12 and folic acid, which act as a co-enzyme for homocysteine metabolism (re-methylation of homocysteine to methionine), or to different reasons for raised aggregate homocysteine [23]. for example, ecological elements or fundamental sickness. Holstein et al. (2011) pointed out that hyperhomocysteinemia and S-adenosylhomocysteine have an effect on bone health. Additionally, a low limit of methylation, apprehension of morphology in diminished bone among peoples. These nutritional elements interpret that there is a relationship between the modified bone morphology and plasma level of homocysteine and S-adenosylhomocysteine. At present, there are limited investigations intended to assess the impact of vitamin B12, folate and vitamin D3 on bringing down plasma homocysteine level toward normal and the consequences for bone comfort.

Vitamin D_3 and Bones

The process of bone mineralization is an important natural mechanism to keep bones safe. There are many physiological factors either directly or indirectly affect such process even positively or negatively. Vitamin D3 (1,25 OH2D), the active form of vitamin D3) is regarded as one of a major substance that keep bone homeostasis, any deficiency in vitamin D3 level will cause bone deformities and bone diseases, for example, osteomalacia, bone loss and development of osteoporosis and bone fractures (particularly in elderly women) [24].

Studies demonstrated that vitamin D3 has a multi effect on bones. The direct role of this vitamin on bones is very complicated process, because it acts directly on certain cell types which are the main bone constituents, for instances, osteoclast, osteoblast and bone stromal cells. There is a direct relationship between vitamin D3 and parathyroid hormone (PTH). Formerly, scientists concluded that vitamin D3 play a role in resorption of bones because of its action on bones through stimulation and differentiation of certain cell types named precursor cells. This is a mononuclear phagocyte of macrophage lineage to be combined with osteoclasts cells (multinucleated and mature cells). Studies concluded that vitamin D3 contribute in the process of early stage of osteoclast production which is called osteoclastogenesis, through which vitamin D3 directly acts on osteoclast precursor cells, which compromises a complex interaction among cells like bone stromal cells, osteoblasts and osteoclast precursor cells [25]. In the late step of the differentiation protocol, the produced osteoclasts will loss its VDR, and then vitamin D3 indirectly promotes the differentiation by acting on cells in osteoblast lineage. Perhaps, osteoblast stromal cells will induce osteoclast differentiating – inducing substances.
The present study will show the great relation between both Homocysteine and vitamin D3. Sufficient vitamin D3 will affect negatively on Homocysteine levels as well as good bone health and vice versa as shown in (Table 3.11). Similar results were obtained by Yuefeng Zho et al (2016), in which patients with elevated homocysteine had deficient to vitamin D3 [25].

Effect of vitamin B complexes on bones

Generally, vitamin B complexes have a great effect in bone preservation particularly vitamin B12. The present study illustrates the positive relationship between this type of B vitamin and homocysteine as shown in table (3.7). The Rotterdam study (2007) believed that bone mineral density (BMD) is associated with osteoporosis. Linking of the collagen to the bone matrix will affect by the circulating homocysteine concentration. However, elevated homocysteine concentration will cause an alteration in bone matrix and resulting fragile bones. Alteration in collagen cross linking will not affect bone mineral density (BMD), therefor, the findings are bone fractures rather than bone mineral density alteration as concluded by van Meurs et . al (2004).

Several researches hypothesized the great effect of B vitamins on bones particularly vitamin B12 as well as its deficiency will cause weaken the maturation process of osteoblast cells. Scientists hypothesized that alkaline phosphatase activity and proliferation of osteoblasts are under control of circulating vitamin B12 concentration, while on the other hand, Herrmann et. Al. (2007) was unable to demonstrate any reliable

and significant association between osteoblast activity and circulating folate and vitamin B12 concentrations. Recently, studies suggested that high circulating homocysteine concentration and low concentration of vitamin B12 will make stimulation to osteoclast activity as well as bone fragility. In spite of folate and vitamin B12, there are another B complex vitamin like vitamin B6 (pyridoxine), micronutrients and choline contribute in homocysteine metabolism as well as affecting plasma homocysteine level and subsequently affect bone health. Because of folic acid and vitamin B12 are the most common substances interfering plasma homocysteine level, therefore, both are regarded as homocysteine – lowering agents.

The impact of folic acid on bones

Folic acid plays a great role in bone health. This will contribute in homocysteine remethylating process to methionine. The present research demonstrates a significant association between folic acid and homocysteine as shown in (Table 3.8). The Hordaland homocysteine study (2007) illustrates that folic acid has been connected to bone mineral density and a reduced fracture, in addition, homocysteine is an indicator for hip fracture among elderly people. Folate was an indicator among women, however there is restricted proof to help a direct unthinking impact of folate on bone [26].

Homocysteine as a marker in Osteoporotic patients (T – Score Quartiles)

The degrees of osteoporosis are varies depending on the T – score value. The degree of osteoporosis was documented by DEXA machine (the gold standard method for diagnosing osteoporosis). According to WHO criteria, osteoporosis is classified into three groups (Mild = -2.5 ~ -3.4, Severe = -3.5 ~ -4.4, Severe with fracture = < -4.5). In the present study, homocysteine was highly significant (p value = > 0.01) with T – score quartiles as showed in (table 3.14) [27].

Statistically the correlation between homocysteine and all study parameters (Age, waist circumferences, body mass index, vitamin D3, folic acid, vitamin B_{12}, calcium, T score and total body fat) are highly significant (*p* value > 0.01) as shown in table (3.19). This correlation means that overweight elderly women with insufficient vitamin D3 (insufficient exposure to sun light or inadequate gaining dietary vitamin D3) also deficiency of each of folic acid and vitamin B_{12} (less than 2 ng / ml and less than 199.9 pg / ml respectively) are more susceptible to elevation of homocysteine level as well as development of osteoporosis, because folic acid and vitamin B_{12} have a great role in homocysteine metabolism and recycling back to methionine [27].

Conclusions

According to the findings of the present study the researcher concluded that:

- The study showed that the majority of the study participants were elderly women and all of them were menopaused, most of them were obese and overweight with abnormal waist circumference accompanied high percent of total body fat (TBF).
- Plasma levels of vitamin D3, vitamin B12 and folate were low in patients with osteoporosis.
- Plasma Homocysteine were significantly high in patients with Osteoporosis. This

was seen particularly in patients with high T – Score values.

- The current study concluded that the majority of the osteoporotic group have high Homocysteine level with very low T – scour values.
- High body mass index (BMI) and waist circumferences (WC), and low vitamin B12, folate and vitamin D3 levels showed high homocysteine levels with low T– scour values.
- There were significant association between age, vitamin status, total body fat or BMI and T – scour values and total plasma homocysteine.
- Finally, the present study conclude that total plasma homocysteine is positively correlated with age, plasma levels of vitamins (folic acid, vitamin B12 and vitamin D3), body mass index and also clearly correlated with menopaused status.

Conflict of Interests.
There are non-conflicts of interest .

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الخلاصة

الخلفية والاهداف: الدراسات الحديثة تشير الى انه المستويات العالية للحامض الاميني هوموسيستين له علاقة مباشرة بزيادة نسبة خطر الاصابة بتنخر العظام التي تؤدي الى الكسور لدى المسنين خاصة النساء (بعد سن اليأس). و تشير ايضا ان مستوى هذا الحمض الاميني مرتبط بشكل اساسي بمستويات كل من فيتامين بي 12, فيتامين دي 3 و حامض الفوليك. زيادة نسبة الهوموسيستين في بلازما الدم وكذلك انخفاض ملحوظ في مستويات كل من حامض الفوليك, فيتامين بي 12 و فيتامين دي 3 يشير الى زيادة احتمالية حدوث هشاشة وفقدان قوام العظام. لذلك, تهدف هذه الدراسة الى تحديد مستوى الهوموسيستين في الدم, ففي حالة زيادة نسبة الهوموسيستين عن الحد الطبيعي (أكثر من 15 μmol/L) وانخفاض مستوى كل من حامض الفوليك, فيتامين بي 12 و فيتامين دي3 تتنبأ بسوء تشخيص حالة العظام و هشاشته وبالتالي الكسور.

طريقة العمل: اجريت دراسة مقطعية على (90) امرأة تتراوح أعمارهن بين (45 – 85) سنة في عامي 2017 – 2018. تم استخدام جهاز (ديكسا) للحصول على المعلومات حول قيمة (تي – سكور) المسجلة من حاسبة الجهاز الموجود في مركز دهوك للامراض الروماتيزمية واعادة التأهيل الطبي.في هذه الدراسة تم استخدام برنامج (اس بي اس اس) الاصدار رقم (23) لغرض الحصول على أنه هل هناك علاقة أحصائية بين اجمالي الهوموسيستين في بلازما الدم و معاملات الدراسة الاخرى.

النتائج: يرتبط فرط الهوموسيستين في الدم بتشوهات الهيكل العظمي وهشاشة في العظام. في هذه الدراسة اختبرنا ما إذا كانت مستويات الهوموسيستين والإنزيمات الحرجة في عملية أيضه مثل فيتامين بي 12 و فيتامين دي 3 و حامض الفوليك التي لهم علاقة بكثافة المعادن في العظام التي تقاس بجهاز ديكسا للمرضى (بعد انقطاع الطمث) المشاركين في هذا البحث. تم الوصول الى النتائج على انه الهمومسيستين على علاقة (p اقل من 0.001) وثيقة مع كل من العمر, قياس الخصر, حامض الفوليك, فيتامين بي 12, وفيتامين دي 3 و كذلك مع مجاميع (تي – سكور) و أيظا له علاقة مع كل من (p اقل من 0.005) مع كل من كالسيوم الدم, مؤشر كتلة الجسم و اجمالي الدهون في الجسم.

الاستنتاجات: يبدو أن الحمض الاميني الهوموسيستين هو مؤشّر على تطور مرض هشاشة العظام بين مجتمع الدراسة (النساء المسنات في مدينة دهوك). البيانات الحالية تشير إلى وجود علاقة كبيرة بين فيتامين بي 12, فيتامين دي 3 و حامض الفوليك وتمعدن العظام. توثق بياناتنا الفرضية القائلة بأن الحمض الاميني الهوموسيستين قد يلعب دورًا في التسبب في تطور حالة هشاشة العظام وكسورها.