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Impact of vitamin C level on osteoporosis of postmenopausal Iraqi women

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***Abstract*---**A common disease, Osteoporosis (OP), is characterized by a systemic impairment of microarchitecture and bone mass that causes in fragility fractures. The medical and socioeconomic influences of postmenopausal OP will increase, meanwhile, oxidative Stress (OS) and inflammation are play an important role in this disease. The intracellular antioxidant vitamin (C ;VitC) is important for a variety of biological functions, and prevents of inflammation and OS. The purpose of this study is investigation the impact Vit C in this silent disease. Total of (150) individual samples were included ; 50 individual for each group (apparently healthy individual samples as control group, Osteopenia patients , and OP patients). These three groups were classified according to the T-Score obtained from the DEXA scan. The vitamin was measured by ELISA immunological method while several biochemical parameters including calcium (Ca^{++}), phosphorus (Phos), total protein (TP), and uric acid (UA) were measured using autobiochemical analysis. Results showed significantly decrease in Vit C in two patients groups when compared with control group but the difference was non-significant between the two patient groups. Also Ca^{++} significantly decrease while TP,UA and Phos were significantly increased between the groups of patients when compared with the control group. According to the results, low levels of vit C in the serum contribute to the development of OP by reducing the body's ability to scavenge free radicals, which cause OP.

***Keywords*---**vitamin C, osteoporosis, osteopenia, phosphorus, calcium.

Introduction

OSteoporosis (OP), a skeletal disorder that affects many people, was characterized by increased bone fragility and susceptibility to fracture. It's fractures predominantly occur at forearm level, lumbar spine and hip ; are associated with substantial morbidity and mortality. For example, , in the first year after fracture, hip fractures are correlated with mortality of up to 36% (Hendrickx et al., 2015). Another clinical term (osteopenia) describes a reduction in bone mineral density (BMD) that is below normal reference levels but not low enough to be considered OP (Varacallo et al., 2021).Such common bone disease (OP) in humans is a silent disease because a lack of observable symptoms until a fall or an impact of an adequate force results in a bone fracture, the patients are often unaware of this disease progression. Pain as well as other complications can also result from OP like reducing a patient's ability to engage in activities of daily living. Therefore, identifying individuals with increased risk of experiencing a fracture is crucial for early intervention and minimization of fracture risk(Cummings & Eastell, 2020).The goal of OP care is prevention of fractures and ultimately reduction in morbidity and mortality associated with it(Rajan et al., 2020). Overall, OP is three times more common in women than in men, because women have a lower peak bone mass, which is increased by the hormonal changes (the state of an absence of menstrual periods for one year due to the inability of the ovary to release estrogen)causing bone loss and risk factors for OP (Shamsulddin et al., 2020). In Iraqi postmenopausal women, OP was linked to ageing, menopause, and x-ray osteopenia(Alosami et al., 2019). Oxidative stress (OS) is thought to play a role in bone metabolism disorders, especially OP in which higher OS levels are frequently found in OP patients' bone tissue that (ROS) and antioxidant systems may play a role in the pathogenesis of bone loss(Kang, 2012). As a result, OS could be a target for the therapy of OP (Li et al., 2020; Mohamad et al., 2020). Disease acuteness and bone mass loss are linked to increased inflammation-stimulating cytokines systemic inflammatory disorders (Qasim et al., 2021).

L-ascorbic acid is a water-soluble micronutrient vitamin (Vit.C) that required for multiple biological functions. It is necessary for normal growth and development(Pehlivan, 2017). A powerful antioxidant vitamin (Vit C) improves endothelial function and microcirculatory flow while modulating the immunological response through various routes(Hwang et al., 2020). It may aid in the resolution of inflammation, OS, and microvascular dysfunction more quickly(Langlois et al., 2019), it has been proven to protect cells from free radicals, thus reducing OS(Erkan et al., 2021) by radical scavenging activity is one electron transfer reaction. (Deshmukh & Kim, 2019). The goal of this study was to determine how serum vitamin C levels impacted osteoporosis in serum of aging (postmenopausal) Iraqi women .

Materials and Methods

The current study was conducted on 150 postmenopausal Iraqi women divided (43-75 years old) into three groups. First group (n= 50) were healthy individuals, second group (n= 50) were patients with Osteopenia while the third group (n= 50) were patients with OP. The subjects were divided according to the T-score for bone mineral density obtained from the DEXA scan. The patients' samples were

collected and diagnosed by Osteoporosis Department in Baghdad Teaching Hospital in the medical city complex in the period from October to December 2021. The Ethics Committee of the College of Sciences, University of Baghdad, Iraq approved the study procedure. From all participants five to ten millilitres of venous blood were taken, placed in gel tubes and left for 20 minutes at 25^{0}C. After clot formation, centrifugation had undergone (3,000 cycles/min for 5 minutes) to obtain serum. The serum was put in to an Eppendorf tube and stored in -20 ℃ freezer until it was analyzed. Laboratory tests include Ca^{++}, Phos, TP, and UA measurement by using autobiochemical analysis. Measurement of VitC using an ELISA immunological method.

Results

The study included 150 postmenopausal Iraqi women, and the samples were divided according to the T-score as shown in the Figure1. Where there were significant differences between the study groups.

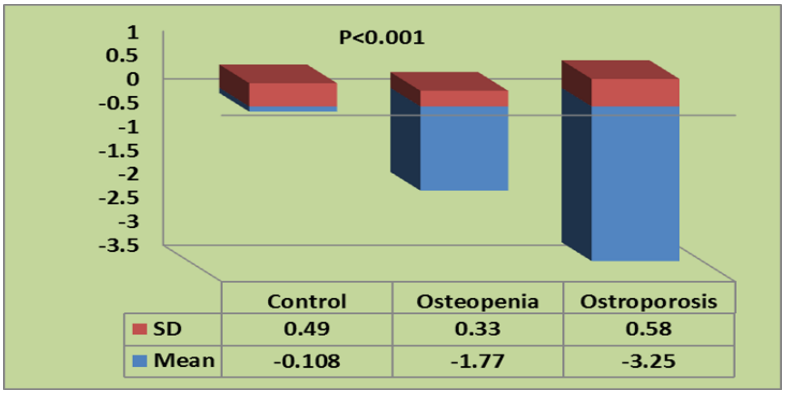

Figure 1: The mean± SD of T- score between the study groups

The above Figure indicated that the T- score value was decreased (significantly)between the study groups. Where a T-score of -1.0 to -2.5 signifies osteopenia, meaning below-normal bone density without full OP. While a T-score of -2.5 or lower qualifies as OP (Salman & Ahmed, 2020). The results of the comparison of Osteopenia and OP with the control group are described in Table 1.

Table1:(Mean±SD) of clinical characteristics of the studied groups

Parameter	Control Group (Mean ± SD)	Osteopenia group (Mean ± SD)	Osteoporosis group (Mean ± SD)	Comparison of Sig.	
				p value	Sig
Vit C (µg/ml)	8.18 ± 3.91	2.81 ± 0.78^b	2.73 ± 0.94^a	< 0.001	S
TP (g/dl)	4.47 ± 1.19	6.45 ± 1.20^b	6.70 ± 1.56^a	< 0.001	S
UA (mg/dl)	4.71 ± 0.82	7.52 ± 0.99^b	7.52 ± 1.09^a	< 0.001	S
Ca^{++} (mg/dl)	10.02 ± 0.83	7.91 ± 1.81^b	8.14 ± 1.96^a	< 0.001	S
Phos (mg/dl)	3.24 ± 0.48	4.52 ± 0.61^b	4.30 ± 0.44^a	< 0.001	S

(a): indicated significant difference between Osteoporosis and Control
(b): indicated significant difference between Osteopenia and Control

The results appeared a significant decrease in VitC, and Ca++ of osteopenia as well as OP when compared with control. While the TP, UA, and Phos levels increased significantly in both patient groups when compared to the control group. No significant differences were observed between Osteopenia and OP, As shown in the above Table. In statistics, dependence or correlation is any statistical relationship (causal or not) between two random bivariate data or variables Figures 2and 3 shows correlations between Vit C and Phos in control, Vit C and UA in OP group, respectively.

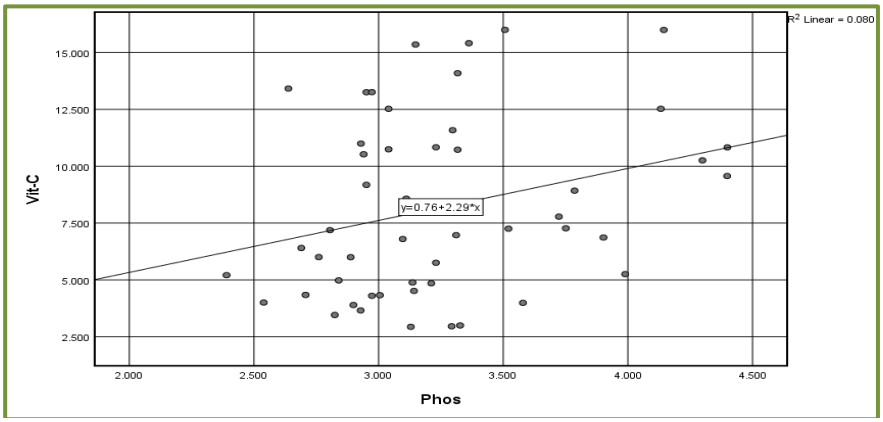

Figure 2: Correlations between Vitamin C and Phos in Control group

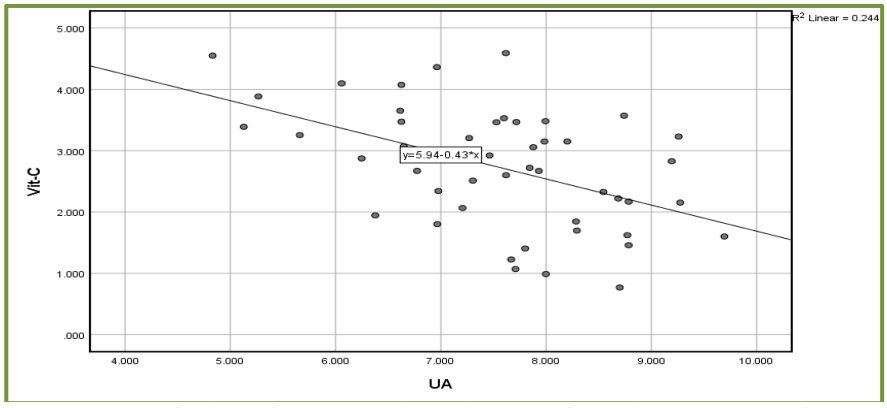

Figure 3: Correlations between Vitamin C and UA in osteoporosis group

From the correlation result, Figure 2 and 3 appears that there is positive correlation between VitC and Phos in control group(P-value 0.283*)(r=0.046), while strong negative correlation with UA in OP group(p-value -0.493**)(r=0.000).

Discussion

World Health Organization (WHO) described the silent disease ;OP by the as a "progressive systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture(Shamsulddin et al., 2020). The

intracellular OS increases osteoclast bone resorption while inhibiting osteoblast bone formation when associated with inflammatory cytokines(Lin et al., 2019). The results appeared to show a significant decrease in VitC of osteopenia as well as OP when compared with control. Vit C is known as ascorbic acid (AA), which is the reduced form of Vit C. It is essential for the normal physiological functions of the body(Aruoah & Al-Jowari, 2022). It scavenges free radicals by forming the ascorbyl radical, which protects macromolecules like lipids and DNA from oxidation. When two ascorbyl radicals are dismutated, one ascorbate molecule and one DHA molecule are produced (Hill et al., 2018). Most immune cells retain high amounts of vit C (ascorbate), influencing several elements of the immune response (Ang et al., 2018). This water soluble vitamin acts as an antioxidant, lowering the levels of ROS, which promote bone resorption. Furthermore, it promotes osteoclast differentiation. According to Kim et al., postmenopausal women with OP consume less vit C than women with normal bone mineral density(Kim et al., 2015). Concerning TP, its concentration was increased significantly in both patient groups when compared to the control group. Proteins are essential components of all cells, and they are found in all bodily fluids. They play a crucial role in cell structure and function(Mallikarjunappa & Prakash, 2007). Increased protein consumption may have a negative impact on bone health. This is based on the "acid-ash hypothesis," which states that animal proteins are acidic and can cause pH disturbances in the body. Because a balanced pH is required for the successful functionality of all body cells, the body will counteract an acidic state by increasing the availability of alkaline minerals, thereby restoring pH equilibrium. The problem is that the majority of the body's alkaline minerals (for example, calcium) are kept in the bones. Long-term bone mineral loss and weakening can occur due to a continuous requirement to normalize pH in response to routinely excessive protein consumption. As well as the increased calcium found in the urine after consuming protein (Macdonald et al., 2005).Some have suggested that overeating protein can induce calcium loss, resulting in weaker bones. There is a greater risk of fractures or OP due to increased urine calcium excretion with excessive protein intake. Urinary calcium levels rise as protein intake increases, with most people developing a calcium deficiency (Heaney & Layman, 2008). The immune system is closely connected to bone metabolism (Al-Masaoodi et al., 2019), inflammatory cells release cytokines into the bloodstream after tissue injury, stimulating hepatocytes to synthesize proteins directly involved in the body's defence processes (Charlie-Silva et al., 2019). Levels of UA increased significantly in Osteopenia and OP groups when compared to the control group.

The correlation between UA and OP is most likely due to uric acid's antioxidant capacity. Some studies, however, have found no link between UA and OP or osteoporotic fractures. Due to conflicting findings of earlier experimental and clinical studies on UA's impact on OP and the oxidant-antioxidant paradox of UA, the role of UA in bone metabolism warrants further investigation (Yan et al., 2018). There have been conflicting results on the possible association between UA and bone metabolism. Urate crystals have been shown to promote osteoclast development and reduce osteoblast activity *in vitro*, suggesting that UA negatively affects bone metabolism(Veronese et al., 2015). The circulation of cytokines and ROS is induced by UA. Through xanthine oxidase (XO), cytokines cause OS, which leads to increased bone loss. Inflammatory cytokines and OS both increase

osteoclast activity while decreasing osteoblast function, resulting in bone loss . Because OS and inflammatory cytokines can promote bone resorption and decrease bone formation in hyperuricemia, the UA raises the risk of fracture, This is consistent with our study(Lin et al., 2019). The results show a significant decrease in Ca^{++} of osteopenia as well as OP when compared with control. While the Phos levels increased significantly in both patient groups when compared to the control group. OP is a condition in which the bones become weak and easily broken. Ca^{++}, which is considered the major component of the bones, causes metabolic disorders that affect the metabolism of the bones. A decrease in the amount of Ca^{++} in the blood is caused by a decrease in the amount of Ca^{++} in the diet as well as factors that limit absorption from the intestine. The body adjusts for the deficiency by absorbing Ca^{++} from the bones, causing them to weaken. As a result, Ca^{++} in the OP group is lower than in the control group(Salman & Al-Shammaa, 2021). The body's Ca^{++} and Phos levels react in opposite ways. Phos levels fall when blood Ca^{++} levels rise (Strause et al., 1994). Several researchers have focused on the role of these two parameters as supplementary compounds. According study in Iraq found that Phos levels in postmenopausal women are considered important for diagnosing OP. This was in agreement with our study(Al-Khakani et al., 2018). In a 2017 study, Zhou, P. et al. explained that evaluating levels of bone metabolism markers such as Ca^{++} and Phos cannot reflect the actual BMD status of perimenopausal women. To diagnose patients' bone loss, bone markers and bone scans must be used together (Zhou et al., 2017). No significant difference was observed between Osteopenia and OP may be Because OP is an advanced condition of Osteopenia, it has similar clinical effects. The correlation results in Figure 2 show a positive correlation between Vit C and Phos in control group. Bone tissue is 90% made of collagen, and Vit C is a necessary antioxidant for its synthesis. Moreover, it is possible that ascorbic acid participates in the synthesis of osteoblasts and influences osteoclast differentiation . In fact, long-term Vit C supplementation was connected with a higher BMD in 55 to 64-year-old women. The human body contains about 700 g of Phos , which is mainly stored in bones (80–90%). Hence, both its excessive and inadequate intake can develop OP(Ratajczak et al., 2020) . However, a high intake of Phos, specifically when it is associated with a low intake of calcium, can be harmful. In contrast, an adequate intake of Phos is essential for bone formation during the growth ages because low serum Phos levels limit the formation and mineralization of bone(Hejazi et al., 2020). While negative correlation between Vit C and UA in osteoporosis group. The circulation of cytokines and ROS is induced by UA Bone loss is caused by a decrease in osteoblast cells and an increase in osteoclast cells (Lin et al., 2019). UA, is also related to the production of ROS like superoxide and hydrogen peroxide during the conversion of hypoxanthine to xanthine and xanthine to UA . When UA is present in a hydrophobic environment, it acts as a pro-oxidant, which can result in OS and inflammation (Ristic et al., 2020). While Vit C, a powerful antioxidant, an enzyme cofactor, may aid in the resolution of inflammation, OS, and microvascular dysfunction more quickly(Langlois et al., 2019) . It has been proven to protect cells from free radicals, thus reducing OS (Erkan et al., 2021). Total Vit C intake was linked to a lower UA level in the blood. Vit C supplementation at 500 mg/d for two months reduced serum UA by 0.5 mg/dL(Gao et al., 2008).

Conclusion

According to our findings, vit C deficiency can increase the development of OP. This is because vit C functions as an antioxidant that can scavenge free radicals, which are one of the leading causes of OP. As a result, vit C supplementation may have therapeutic efficacy in reducing OP. When vit C is included in the diet, OP can be delayed.

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