Advances in Pharmacology and Pharmacy 6(2): 43-49, 2018 http://www.hrpub.org
DOI: 10.13189/app.2018.060202

Effect of Quercetin Supplement on Some Bone Mineralization Biomarkers in Diabetic Type 2 Patients

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Abstract Background: Diabetes associated with multiple metabolic problems in the body, including bone mineralization remodeling, osteoporosis and increase risk of fracture. Quercetin is natural flavonoids and according to animal studies; it has potent antioxidant, antidiabetic and protective effect against bone loss due to various causes. Objectives: explore effect of quercetin as nutritional supplement administrated orally on some bone mineralization bio-markers such as calcium, vitamin D and osteocalcin in Iraqi diabetic patients. Methods: interventional double-blind placebo randomized controlled study in which 40 patients with type 2 diabetes mellitus (age range 40-45) assigned randomly (using simple randomization) in either control (n=20) or study (n=20) group. Study group received Quercetin oral supplement as 500mg capsule once daily for three months. Venous blood was used for measuring Serum calcium, 25(OH) vitamin D and osteocalcin at base line and after 3 months. Results: After 3 months treatment with Quercetin; levels of Osteocalcin (28.1±7.6), serum calcium (9.2±1.8) and 25(OH) vitamin D (26.6±8.7) were significantly ($p<0.05$) higher than pretreatment values of osteocalcin (24.0±8.6); serum calcium (7.0±2.2) and 25(OH) vitamin D (20.6±7.7) and control values of serum calcium (6.8±2.0) and 25(OH) vitamin D (20.8±7.4), but not Osteocalcin (25.2±9.0). There was also significant correlation between use of quercetin; elevation of serum calcium and osteocalcin ($r= 0.454$; $p= 0.032$), indicating modulation in bone mineralization. Conclusions: Quercetin's use in diabetic patients may elevate Serum level of Calcium; 25(OH) vitamin D and may modulate bone mineralization represented by elevation of osteocalcin.

Keywords Quercetin, Osteocalcin, Diabetes, 25(OH) Vitamin D, Calcium

1. Introduction

Diabetes recently is considered as global health problem that affect large number of people and wide range of ages. Diabetes also responsible for increase morbidity and mortality rates among diabetic people where it related to a lot of complications; incidence of these complications accelerated when there is insufficient control on blood glucose level. These complications are categorized into microvascular or macrovascular [1] in addition to that; Recently evidences suggest that the bones probably may be affected by uncontrolled diabetes [2] and diabetes patients have greater risk for fracture as compared with normal individual at same age [3]. The elevated risk of bones fragility in those patients may be related to many factors like oxidative stress, hyperglycemia, accumulation of advanced glycation end products. These may produce great change in the metabolism inside bones lead to affect their strength and/or their structure [4]; and fast bone loss and development of osteoporosis [5, 6]. Many biomarkers level may be changed due to effect of Diabetes on bones such as osteocalcin; a bone formation marker, and CTX-1, bone resorption marker, were significantly lower in diabetics [7]. Other markers like IGF1 is found to be lower in in postmenopausal women with type 2 diabetes, while Serum concentrations bone formation inhibitor produced by the osteocyte called sclerostin, is elevated [8]. Circulating osteoprogenitor cells are; a novel bone metabolism marker [9] is measured by flow cytometry, found at lower level in patients with type 2 diabetes. [9, 10]

Diabetes also may affect bone cells directly; in type 2 Diabetes; in state of excess insulin; there were increases histo-morphometric indices of bone formation by two to three times through stimulates osteoblast proliferation [11], according to some in-vitro studies excess insulin also affect osteoblasts and promotes bone resorption [12] and associated with an increased Bone mineral density and might be related to a lower fracture rate [13].

Medications used for diabetes treatment found have variable effects on bone; in large population-based case-control showed use of insulin cause a no significant elevation in risk of any fracture [14]. Metformin effect was

controversial between beneficial effect [15] or no change in risk of fragility [16]. Although Metformin found affects bone metabolism positively by invitro study; where metformin activates AMP-activated protein kinase in bone marrow progenitor cells and primary osteoblasts. [17]

Use of rosiglitazone and pioglitazone; Thiazolidinedione's insulin sensitizers; use in women may leads two times increased risk of fracture [18] where these drugs stimulate PPAR-γ enhances osteoclastogenesis and promote differentiation of mesenchymal lineage cells to adipocytes, impairs osteoblast function may leading substantial negative effects on bone health [19].

Sulfonyl urea effect was similar to metformin in concern of bone fragility [16, 20] Remaining anti diabetic drug classes; their effects were either neutral or reduce risk of fracture [21-25] and other with increase this risk [26].

Quercetin, is a flavonoid naturally available in many fruits and vegetables, is considered as one of potent antioxidants. It used newly in food supplements due to expected health beneficial effects. These include either protection against or amelioration symptoms of various diseases such as heart failure; hypertension, asthma, certain forms of cancer, and fight against aging. The quercetin's ability to stop damaging effects induced by free radicals; that characterized by high reactivity, such as peroxynitrite and the hydroxyl radical, may be involved as one of mechanisms responsible for these possible beneficial health effects. [27]

Quercetin also may have antidiabetic effects that have been demonstrated both in vitro and in vivo studies; these include ability to: reduce blood glucose, promote the regeneration of Langerhans islets and increase insulin release. [28] According to some in vitro studies using animal models; Quercetin may have protective effect against bone loss due to various causes. It may inhibit bone resorption [29] and stimulate bone formation indicated by increase bone density and elevation of bone formation biomarkers like osteocalcin [29-31]. A lot of data presented about roles of quercetin in maintain human health and fighting of common health problems in modern life like Diabetes, osteoporosis and cardiovascular disease are obtained either from invitro studies or in vivo studies using surrogated animal models. There no direct clinical trial conducted in human, even in small scale, to evaluate these effects. [32]

The ***novelty*** of this study is first one describes effect of quercetin as supplement administrated orally on bone biomarkers in diabetic patients.

Aim of Study

It is to explore the effect of quercetin as nutritional supplement administrated orally on some bone mineralization bio-markers such as calcium, vitamin D and osteocalcin in Iraqi diabetic patients.

2. Subjects and Methods

Study Design

Randomized double-blind placebo-controlled study; the study consisted of two groups one is control and the other is study group.

The study was carried out from February 2016 to march 2017 in governmental Al Basra General Hospital, Basra city – southern of Iraq after agreement of scientific and ethical committees in college of pharmacy and hospital. Agreement No. S. A. 234 on 12th Jan. 2016.

Patients Selection

Inclusion Criteria

Adult patient; with age range 45-50 years old, diagnosed with diabetes Mellitus type 2; and each patient used medical diabetes treatment at least for two years.

Exclusion Criteria

Diseases include malignancy, thyroid problems; parathyroid; pregnancy or breastfeeding, medications' use; like vitamin D; calcium supplements; anti-obesity medications or blends; steroids, bisphosphonates and insulin at least one month before starting study and for the next 3 months of study.

Sample Size Determination

Was determined using PS® software assuming 1:1 subject division (control: study) the response within each subject group was normally distributed with standard deviation 2. If the true difference in the study and control means is 2, we will need, at least; to study 19 subjects for study and 19 control subjects to be able to reject the null hypothesis that the means of the study and control groups are equal with probability (power) 0.85. The Type I error probability associated with this test of this null hypothesis is 0.05.

Study Groups

117 diabetes were evaluated in outpatient diabetic clinic of the hospital. Only 40 diabetic patients were fulfilled the requirements of the study and accept to participate and sign the written consent and randomly assign; using simple randomization, into one of study groups.

Control group (n=20, age 47.2 ±1.96 years; 11females & 9 males) received placebo treatment once daily and

Study group (n=20, age 46.9 ±1.77 years; 12 males & 8 females) received Quercetin 500mg capsule (Jarrow formulas) orally once daily. There was no significant difference in average ages and male: female ratio between groups.

Each patient gets full information about the goals of the study and full information about the Quercetin by the hospital's pharmacist. Each patient included in the study after signing of a written consent.

Blood Sampling and Lab Data Measurements

A venous blood sample was drawn from each participant, for measuring random blood glucose; serum calcium; Osteocalcin; 25-(OH) Vitamin D level; before and three months after initiation of study.

Biochemical indices were measured using specific kits as in the table 1.

Table 1. Show name and source of kits used to measure the parameters of study

parameters	Kit	Source
Random blood glucose	Glucose Assay Kit (Colorimetric)	Cell Biolab, INC
serum calcium	Calcium Assay Kit	BD biosciences, USA
Osteocalcin	Osteocalcin (1-43/49) ELISA	ALPCO diagnostics
Serum 25-OH-Vitamin D	25-OH-Vitamin D direct ELISA	IBL INTERNATIONAL GMBH

Data Analysis

Data analyzed using Medcalc ® software V12. The data was expressed as mean ± Standard deviation.

Independent sample t-test was used for comparison between groups and paired t test was used to find the significant of difference between pre-and after treatment values within each group.

P value < 0.05 was considered as significant.

3. Results

Demographic Data of Patients

As in table 2 there were no significant ($p<0.05$) differences between control and study group. In age; male: female ratio; weight (kgs); Height (cm), Body mass index; obesity ratio and Baseline Blood glucose.

Table 2. Demographic data of patients in the study groups; some of data expressed as Mean ± standard deviation

	Control group n=20	Study group n=20	P values
Age (years)	47.2 ± 1.96	46.9 ± 1.77	0.615
Male: female ratio	9:11	12:8	0.527
Weight (kg)	95.4 ± 11.97	96.3 ± 11.96	0.813
Height (cm)	168.8 ± 7.98	171.1 ± 6.25	0.306
Body mass index (kg/m^2)	33.6 ± 4.76	32.9 ± 4.12	0.627
Obesity ratio	15 (75%)	16 (80%)	1.000
Random Blood glucose (mg/dl)	210.5 ± 55.3	208 ± 56.7	0.888
P values<0.05 considered as significant values			

2-Bones Mineralization Markers

Osteocalcin was elevated significantly ($p<0.05$) in study group; after 3 months treatment with quercetin as compared with pre-treatment value. This elevation was not significant as compared with control value after treatment. As in table 3

Serum Vitamin D was elevated significantly ($p<0.05$) in study group; after 3 months treatment with quercetin as compared with pre-treatment value. This elevation was also significant as compared with control value after treatment. As in table 3

Serum calcium was elevated significantly ($p<0.05$) in study group; after 3 months treatment with quercetin as compared with pre-treatment value. This elevation was also significant as compared with control value after treatment. As in table 3

Table 3. Pre-treatment and after 3 months treatment values of control and study groups; Data expressed as Mean ± standard deviation

	Pre-treatment		**After 3 months treatment**	
	Control group (n=20)	**Study group (n=20)**	**Control group (n=20)**	**Study group (n=20)**
Osteocalcin (ng/ml)	23.6 ± 9.2	24 ± 8.6	25.2 ± 9	28.1 ± 7.6*
Serum Vitamin D (ng/ml)	20.1 ± 7.6	20.6 ± 7.7	20.8 ± 7.4	26.6 ± 8.7*a
Serum Calcium (mg/dl)	6.8 ± 2	7 ± 2.2	6.5 ± 2	9.2 ± 1.8*a
* significant (p value<0.05) as compared with pre-treatment values a Significant (p value<0.05) as compared with control values				

Correlation of Measured Parameters

***Osteocalcin*;** a bone formation biomarker, was not significantly correlated with *serum vitamin D*; but it was significantly correlated with serum calcium.

Serum calcium and vitamin D were not significantly correlated in this study. As in table 4.

Table 4. Correlation coefficients of parameters measured in the study

	Osteocalcin		Serum 25(OH)Vit. D		Serum calcium	
	r -value	P value	r -value	P value	r -value	P value
Osteocalcin (ng/ml)	1.000	1.000	-0.410	0.058	0.454	0.032*
Serum Vitamin D (ng/ml)	-0.410	0.058	1.000	1.000	0.031	0.895
Serum Calcium (mg/dl)	0.454	0.032*	0.031	0.895	1.000	1.000
* (p value<0.05) and consider significant						

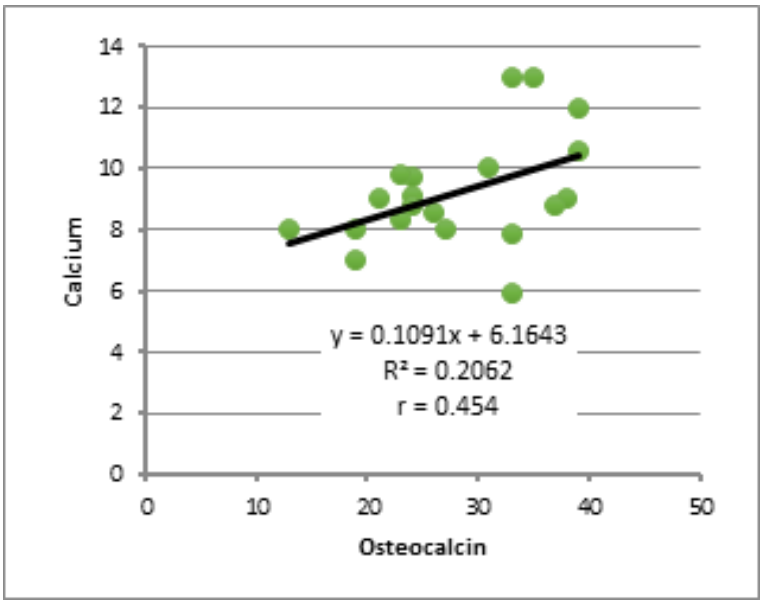

Figure 1. Scattered diagram show correlation of serum calcium with osteocalcin; The correlation was significant

4. Discussion

Uses of flavonoids, such as Quercetin, in medicine recently expanded largely depending findings from in vitro and in vivo studies. Where findings from these studies showed these chemical substances have capability to fight many diseases including diabetes, cancers, cardiovascular, osteoporosis and other human diseases. Most of these chemicals are used as nutraceutical. [33] Quercetin, in this study; when used for 3 months was able to rise level of osteocalcin significantly ($p<0.05$) in study group. This finding was in agreement with Wei Liang & et al's; 2011 study in which serum level of osteocalcin was elevated significantly in study group of diabetic rats, where quercetin was given in concentration of 30-50mg/kg [34].

Our study was also in agreement with finding of Hoda Derakhshanian & et al's in 2012 study where they reported that blood level of osteocalcin in rats was significantly increased after use of Quercetin for study of bone metabolic complications resulted from biliary cirrhosis indicating improving bone strength [35]. As in table 3; use of quercetin led to elevation of serum calcium, this finding

is similar to finding of Marwan A. & et al in 2012, where treatment of rats with quercetin resulted in elevation of serum calcium as compared with group with gentamicin nephrotoxicity [36]. The mechanism behind elevation of serum calcium in our study is not well understood, but may be related to potent antioxidant effect of quercetin that may correct redox status in small intestine at cellular level [37]; activation of TRPV6 gene expression and activation of vitamin D receptor in intestine [38] leading to improvement of calcium absorption [39].

The unexpected finding is elevation in level of 25 hydroxy vitamin D in quercetin treated group, this elevation is not related to any supplement of vitamin D during the study; since we ensured that supplement of vitamin D have been never used by the patients during study period otherwise; they would be excluded from study. The causes behind this elevation in serum 25 hydroxy vitamin D is hard to be explained; but, probably use of Quercetin led to either increase intestinal vitamin D absorption or synthesis through induction the expression of hepatic cytochrome [40], other possible explanation is that;

Quercetin induces activation of TRPV6 gene expression, that in turn, lead to the elevate expression level of CYP27B1 and then production of 25(OH) vitamin D alpha hydroxylase; the enzyme required to conversion of 25(OH) vitamin D to more potent Vitamin D metabolite called 1,25di(OH) vitamin D [41]. Both Increment in level of 1,25di(OH) vitamin D and Quercetin mimic effect of 1,25di(OH) vitamin D on its receptor (VDR) [39], both actions lead to elevation in serum calcium, [42] and hence may reduce parathyroid hormone [42], these possible two last changes may reduce conversion of 25(OH) vitamin D to 1,25di(OH) vitamin D and then increase serum level of 25(OH) vitamin D.

There was strong association between increase in the osteocalcin and elevation in serum calcium after quercetin use (table 4) this may indicate modulation in bone mineralization [43]; where osteocalcin is one of calcium- and phosphate binding protein such as sialoprotein and osteopontin, that have role in bone mineralization through by regulating minerals deposition, size and amount of hydroxyapatite crystals formation in bones. [44]

5. Conclusions

Quercetin use in diabetic patients may have many favorable effects that discussed in several published articles. In our study we found that supplement of quercetin 500mg capsule orally as single daily dose for three months was sufficient to elevate Serum level of Calcium; 25(OH) vitamin D and may modulate bone mineralization represented by elevation osteocalcin. So Quercetin supplement could be helpful for diabetes type 2 patients for fighting the osteoporosis.

Limitation of Study

This study needs evaluation of additional biomarkers or use Bone density report (Dexa Scan) for determining how much effect of quercetin supplement on bone mineralization.

Acknowledgements

To all medical staff of Basra general hospital who assist us in accomplishing this study.

Conflict of Interest

There is no conflict of interest; and the research is self-funded by researcher himself.

This original article and the trial were approved by scientific and ethical committee in college of pharmacy-university of Basra.

All Patients in this trial agreed to go within trial after explaining the purpose of trial and side effects of drugs used and then asked them to sign the written consent**.**

Source of Funding

The research was self-funding by authors themselves

Author Contributions

Authors are equally contributed in the research and article preparation.

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