

## The Assessment of Some Serum Biomarkers of Fibromyalgia Syndrome in Pre and Postmenopausal Women in Relation to Obesity

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### Abstract

The objective of this study is to evaluate the level of parathyroid hormone (PTH), Co enzyme Q (Co Q) and total antioxidant status (TAS) in serum's women with fibromyalgia syndrome firstly, then to assess the influence of age and obesity on these biomarkers.

This study was performed at Rheumatology and Rehabilitation Consultation Unit in Baghdad Teaching Hospital. Blood sampling from (59) women with fibromyalgia syndrome (FMS) and (30) control be taken. The mean age of FMS group was (42.22±15.34) years and for control was (40.7±18.22 years) and body mass index (BMI) for patients and control were (31.83 ± 5.05 and 30.09± 4.87) correspondingly. Those participants were subdivided into four different groups according to menopausal status; FMS before menopause were (30.14±10.58 and for control were 29.74± 12.21) years, post menopause FMS age were (53.2 ± 16.18 and control were 47.8 ±12.51) years and BMI to measure serum (PTH and Co Q) by enzyme linked immune sorbent assay (ELISA) and (TAS) by spectrophotometry. Results analyzed by SPSS version 24, independent samples T-test and Pearson's correlation, P values < 0.05 were considered significant.

The results record increment of serum's PTH levels significantly, decrement of Co Q (P value<0.05) and non-significant decreased TAS (P value> 0.05) in FMS comparing with control group. Considering the age; PTH serum level showed insignificant increase and decrease of (Co Q & TAS) (P value >0.05 & P value < 0.05) respectively in post menopause comparing with pre menopause. According to BMI; serum PTH level record a significant raise in obese group (P value <0.003), reduction of serum Co Q significantly (P value < 0.05) and insignificantly for TAS (P value > 0.05).

The mean serum level of PTH before menopause correlate significantly with TAS (P value= 0.034) also with Co Q of both FMS and control groups when BMI>30 (P value= 0.029 and P value= 0.043) respectively. In post menopause of both (FMS and control) PTH correlate positively (P value=0.05).

Independent T- test for pre and post menopause FMS showed significance variance in TAS only (P value=0.004). Independent T- test for obese and non-obese FMS showed significant variance in PTH and TAS (P value=0.032 and P value= 0.0001).

In conclusion the obesity and menopause play an imperative role in the etiology of FMS relative with serum's biomarkers (PTH, Co Q and TAS).

**Keywords:** Obesity, Menopause, Fibromyalgia syndrome, Parathyroid hormone, Total anti-oxidant status, Co enzyme Q.

### تقدير بعض المؤشرات الحيوية في مصلى النساء قبل وبعد سن اليأس في متلازمة ألم الليف العضلي وعلاقتها بالسمنة

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#### الخلاصة

وضعت الدراسة لتقييم مستوى هرمون الجار الدرقي، الانزيم المشاركون وحالة مضادات التأكسد الكلية في النساء العراقيات المصابات بمتلازمة ألم الليف العضلي اولا ولبين تأثير العمر ومؤشر كتلة الجسم عليهم. تمت الدراسة في شعبة امراض الروماتيزم والتأهيل الطبي التابعه لمستشفى مدينة الطب بسحب نموذج من دم المريضاات البالغ عددهم (٥٩) اضافة الى (٣٠) نموذج من مجموعة السيطرة. معدل العمر المنحرف القياسي للمريضاات وغير المريضاات هو ٤٢,٢٢ ± ١٥,٣٤ و ١٨,٢٢ ± ٤٠,٧ سنة على التوالي. تم تقسيم المرضى والاصحاء الى اربعة مجاميع حسب العمر وكتلة الجسم، معدل اعمار المريضاات والاصحاء قبل سن اليأس (٣٠,١٤ ± ١٠,٥٨ و ٢٩,٧٤ ± ١٢,٢١) سنة وبعد سن اليأس (١٦,١٨ ± ١٢,٥١ و ٤٧,٨ ± ١٢,٥١) سنة تم قياس الهرمون والانزيم في المصل بطريقة مقايسة الممتز المناعي المرتبط بالانزيم (الأليزا) بينما مقايسة مضادات الاكسده بطريقة التعيين اللوني.

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ظهرت النتائج زيادة معنوية في مستوى الهرمون الجار الدرقي بالمصل ونقصان معنوي للأنزيم ولامعنوي لمضادات الأكسدة بالنسبة للعمر عند مقارنة مجموعتي (المريضات مع السيطره). لوحظ زيادة غير معنوية للهرمون وقله للأنزيم, نقصان معنوي لمضادات الأكسدة عند المقارنة بين قبل و بعد سن اليأس. بينت النتائج هناك زيادة معنوية عند النساء البيديئات المصابات بالمتلازمة عند مقارنتها بمجموعة السيطره لكن الاختلاف غير ملموس في مجموعتي البيديئات وغير البيديئات. وأيضا سجلت حالة مضادات الأكسدة قله معنوية في مجموعة البيديئات فقط. وضحت النتائج هناك علاقة معكوسة معنوية بين مستوى الهرمون الجار الدرقي وحالة مضادات الأكسدة الكلية (P value= 0.034) في مصل مجموعتي المريضات والأصحاء وايضا مع الأنزيم ف (P value= 0.029 and P value= 0.043) بالاعتماد على مؤشر كتلة الجسم لاكثر من 30. ايضا هناك علاقة معنوية حرجه (P value=0.05) في قياس الهرمون بين المجموعتين اعلاه بعد سن اليأس. علاقة التباين بأختبار تي لما (قبل ٤٥ وبعد ٥٥) سنة بينت وجود علاقة معنوية لمضادات الاكسدة للمريضات فقط. وجدت علاقة معكوسة بين الهرمون والانزيم للمريضات البيديئات وغير البيديئات اضافة الى وجود تباين واضح بالهرمون ومضادات الاكسدة لكلا المجموعتين (P value=0.032 and P value= 0.0001)

الكلمات المفتاحية: السن، سن اليأس، متلازمة التهاب النيف العضلي، الهرمون الجار الدرقي، حالمو مضادات التأكسد الكلية، الأنزيم المشارك ف.

## Introduction

Fibromyalgia syndrome (FMS) is a chronic disorder which has been defined by a history of widespread pain and the presence of marked tenderness to palpation at standard anatomically-defined tender points <sup>(1)</sup>. Fibromyalgia (FM) was recognized as a true syndrome with the publication of the American College of Rheumatology (ACR) classification criteria in 1990, which were updated in 2010 <sup>(1,2)</sup>.

The parathyroid glands maintain the serum levels of calcium (Ca) and phosphorus (P). Calcium plays a dynamic role in metabolic processes <sup>(3)</sup>. There is a large symptom overlap between hyperparathyroidism (HP) and FM so the subjects experience “**fatigue**, musculoskeletal pain, headache, cognitive dysfunction, and mood disturbance” <sup>(4)</sup>.

Coenzyme Q (Co Q) or Q<sub>10</sub> is a small fat-soluble, vitamin-like substance located in mitochondria that transfers reducing equivalents from complexes I and II to complex III of the respiratory chain. Some chronic disease conditions are also thought to either reduce the biosynthesis and/or increase the demand for Co Q in the body, but there are no definite data to support these claims <sup>(5,6)</sup>.

Reactive oxygen species (ROS) is produced in metabolic and physiological processes. Under certain conditions, the increase in oxidants or decreased in antioxidants that cannot be prevented, and the disorders related to oxidative stress would develop. Antioxidant molecules prevent or inhibit these harmful reactions. The antioxidant system consist of enzyme and non-enzyme anti-oxidants. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition <sup>(7-9)</sup>. The definition of TAS is the sum total of endogenous and food derived antioxidants of the extra cellular fluid of an individual <sup>(10)</sup>.

## Aim of study

To estimate serum biochemical markers {parathyroid hormone (PTH), Co Q and total antioxidant status (TAS)} in women with FMS and evaluate the effect of age and obesity on these biomarkers.

## Subjects, materials and methods

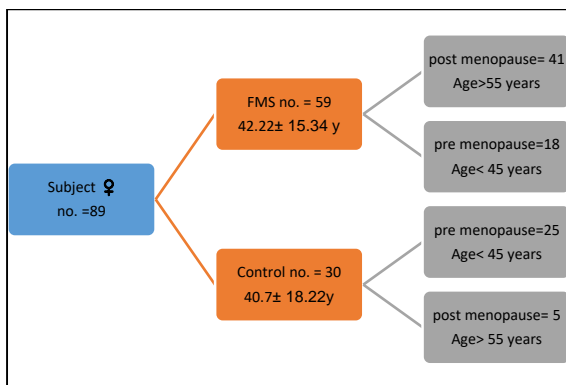
The work was started in September/ 2014 to January/ 2015 at Baghdad Teaching Hospital / Rheumatology and Rehabilitation Consultation Unit, by the assortment of women with FMS which was accomplished under observation of rheumatologist according to American College of Rheumatology (ACR) criteria <sup>(11)</sup>. In this study, blood sample were withdrawn from (59) females with FMS and (30) control subjects without FMS in order to obtain the serum after centrifugation of the clotted specimens. All applicants were classified consistent with their menopausal status before 45 years refer to pre menopause and after 55 years be in post menopause as shown in (figure 1). Note: Each subject must follow above condition. The mean age of FMS before menopause were (30.14±10.58, control were 29.74± 12.21) years; the post menopause FMS were (53.2 ± 16.18 and control were 47.8 ±12.51) years. Determination of (BMI) was by dividing body weight in kilograms over the height in meters square {BMI = Weight (kg) / Height (m<sup>2</sup>)} <sup>(12)</sup> (figure 2).

The assessment of serum PTH (Demeditec Diagnostics GmbH/Germany) and Co enzyme Q (Cusabio Biotech Co., Ltd, /China) were done by enzyme linked immune sorbent assay (ELISA); this assay employs the competitive inhibition enzyme immunoassay technique. The colorimetric method Randox kit used to assess total antioxidant status (TAS) <sup>(13-15)</sup>. Inclusion and exclusion criteria for subjects selected to participate in the study was shown in table 1.

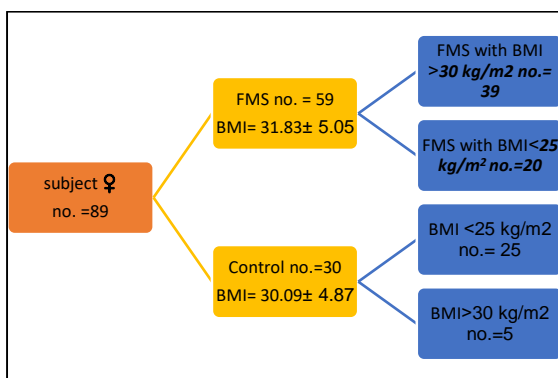
**Table 1. the selection criteria for FMS and control women**

Inclusion criteria	Exclusion criteria
The age range of subjects 20-60 years. The mean age (M) ±standard deviation of mean (SD) of the patients was (42.22± 15.34) years and the control group was (40.7± 18.22) years as well as complete blood picture and erythrocyte sedimentation rate (ESR) tests were within normal range	All women had any endocrinopathiy, autoimmune, inflammatory, rheumatologic disorders, chronic and systemic disease in addition the pregnant and lactating subjects.

Figure 1 refers to distribution of subjects shared this work classified according to their ages less than 45 years as pre- menopause and more than 55 years as post menopause while figure 2 expressed the classification of both FMS& control along with their BMI as normal BMI if less than 25 and obese BMI more than 30.



**Figure1. Demographic distribution of volunteers (FMS and control) according to age in this study.**



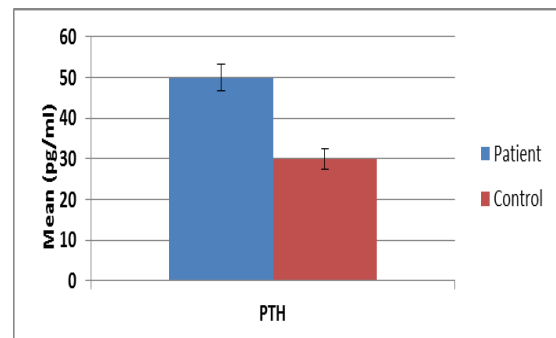
**Figure2.Demographic distribution of (FMS and control) women according to BMI in this study.**

The statistical analysis was done by using SPSS (version 24) for values of measurement of serum biomarkers levels which expressed as mean±SD. Also independent T- test sample were used to complete the analysis and Pearson’s correlation

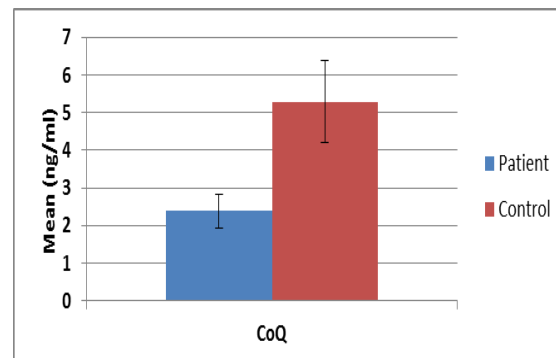
between studied biomarkers in different groups. P value is significant < 0.05.

**The results**

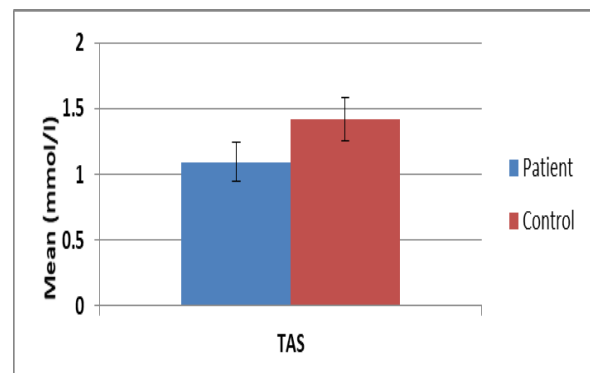
This trial showed a significantly (P value < 0.01) increased serum level of PTH in FMS when compared with control, while the significant decrease of Co Q (P value < 0.01) and insignificant (P value > 0.01) decline in TAS for all subjects as shown in (figures 3,4 and 5, respectively). The mean age ± SD of both FMS and control were (42.22±15.34) and (40.7±18.22) years and BMI were (31.83±5.05) and (30.09 ±4.87) kg/m2 respectively.



**Figure 3. The mean serum PTH level (pg/ml) in FMS and control groups (P value<0.01)**



**Figure 4. The mean serum Co Q level (ng/ml) in FMS and control (P value <0.01)**



**Figure 5.The mean serum TAS level (mmol/l) in both FMS and control groups (P value > 0.05)**

Above groups were discrete into four subdivisions. The first two groups demonstrate the levels of serum biomarkers in FMS and control as post menopause while reminder two groups in pre menopause, these information are listed in table 2. The significant increasing of serum level of PTH in

both age groups with FMS (P value < 0.005), significant decreasing of serum Co Q level (P value < 0.005) and significant decrease in TAS in post menopause group but insignificant in pre menopause groups when compared with control (P value < 0.05 and P value > 0.01 respectively)

**Table 2. The serum level of biochemical markers in both FMS patients and control distributed according to their ages represented by (mean± SD).**

age > 55 years						
	FMS patient no. =41			Control no.=5		
Serum	PTH: pg/ml	CoQ: ng/ml	TAS: mmol/l	PTH:pg/ml	CoQ:ng/ml	TAS:mmol/l
mean± SD	52.15±16.26**	2.36± 0.68**	1.025± 0.06*	30.20±12.51	5.51±2.07	1.39±0.15
age <45 years						
	FMS patient no.=18			Control no.=25		
Serum	PTH:pg/ml	CoQ:ng/ml	TAS:mmol/l	PTH:pg/ml	CoQ:ng/ml	TAS:mmol/l
mean± SD	49.07±24.29**	2.38±0.72**	1.12±0.16	29.74±14.21	5.24±2.63	1.43±0.2

Table 3 shows the effect of obesity on serum biomarkers levels in normal and obese (FMS and control) groups. The results appear that the value of PTH in serum was increased significantly and highly significant (P value < 0.01, P value < 0.005) in both normal and obese respectively when compared with

control groups. The serum level of Co Q shows high significant decreasing in normal and obese FMS groups, insignificant decreased for TAS in comparison with control group (P value < 0.005 & P value > 0.05).

**Table 3. The serum level of biochemical markers (mean± SD) in FMS and control distributed according to their BMI.**

BMI <25						
	FMS patient no.= 20			Control no. =25		
serum	PTH:pg/ml	CoQ:ng/ml	TAS:mmol/l	PTH:pg/ml	CoQ:ng/ml	TAS:mmol/l
mean± SD	35.42±12.04*	2.20±0.85**	1.36± 0.1	27.80±12.89	4.12±0.4	1.72±0.13
BMI >30						
	FMS patient no.= 39			Control no.=5		
Serum	PTH:pg/ml	CoQ:ng/ml	TAS:mmol/l	PTH:pg/ml	CoQ:ng/ml	TAS:mmol/l
mean± SD	52.57±22.65**	2.4±0.68**	1.04±0.09	30.22±14.11	5.51±2.7	1.35±0.14

The mean serum level of PTH before menopause correlate significantly with TAS (P value= 0.034) also with Co Q of both FMS and control groups when BMI > 30 (P value= 0.029 and P value= 0.043) respectively. In post menopause of both (FMS and control) PTH correlate positively (P value=0.05). Independent T- test for pre and post menopause FMS showed significance variance in TAS only (P value=0.004). Independent T- test for obese and non- obese FMS showed significant variance in PTH and TAS (P value=0.032 and P value= 0.0001).

## Discussion

Fibromyalgia syndrome (FMS) is classified as a first-order syndrome or a symptom complex with unknown or unclear etiology, heterogeneous pathogenesis and a defined phenotype; second - order syndromes (sequences) are defined by unknown etiologies, homogeneous pathogenesis and defined phenotypes; Third-order syndromes are defined by homogeneous etiologies, unknown or insignificant pathogenesis and defined phenotypes types<sup>(16)</sup>.

The results show hyperparathyroidism of FMS group if comparing with control so it is in concomitant with the result of Armagan *et al* <sup>(17)</sup> measured serum PTH, calcium, phosphorus and active vitamin D showing in significant increase of PTH. Another study done by Shaheen *et al* through approved the deficiency of vitamin D in fibromyalgia <sup>(18)</sup>. Ferrari R *et al* studied the rate of prevalence related with primary hyperparathyroidism in widespread and localized musculoskeletal pain were 6.4, 5.5, and 6.1 %, respectively <sup>(19)</sup>. Previous studies <sup>(20, 21)</sup> show FMS have a predisposition to osteopenia. Measurement Co Q, in this study showed decreasing in serum level of FMS so this clarify the mitochondrial dysfunction of muscle & neural cell which is a part of electron transport chain (ETC), affect the synthesis of ATP pools and finally lead to widespread pain <sup>(22)</sup>. Study on osteoarthritis demonstrated the same results <sup>(23)</sup>. In US <sup>(24)</sup>; Co Q supplements were set as foods not as drug. About TAS and oxidative stress: this study illustrate that the level of TAS was decreased insignificantly because of presence of reactive oxygen species (ROS) in excess result in cell damage and lipid peroxidation <sup>(25)</sup>. Chung *et al* study match with current study in relation with oxidative stress and FMS <sup>(26)</sup>. Turkish authors compare between FMS& obstructive sleep apnea syndrome in assessing anti-oxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase) were significantly lowered at the same time malondialdehyde was increased so this finding like the present study's result <sup>(27)</sup>. The role of oxidants/antioxidants, mitochondrial dysfunction, and autophagy in fibromyalgia was published recently <sup>(28)</sup>.

The effect of age on serum biochemical markers showed that the variation between pre and postmenopausal groups were unclear insignificant but when compare with control group demonstrate significant differences this finding belongs PTH similar to recent study done by Guncha K and Gagan D that showed significant correlation of age with serum PTH in both pre-menopausal and postmenopausal women <sup>(29)</sup> because an impairment of ovarian function in postmenopausal women alter the metabolism of Ca and therefore reduce bone mass <sup>(30)</sup>. Borgia *et al.* demonstrate the role of hyperparathyroidism in musculoskeletal pain of fibromyalgia <sup>(31)</sup>. In correspondence of present study with Cammozi *et al* and Safi *et al* observations that parathyroid hormone was significantly low in postmenopausal women <sup>(32,33)</sup>. Eastell *et al* and Khosla *et al* in different studies described the progress of age lead to increase turnover of bone due to raise the blood level of parathyroid hormone and lower estrogen <sup>(34, 35)</sup>. The role of estrogen in inhibiting interleukin six was decreased with age so lead to prolongation of osteoclast's life span <sup>(36)</sup>. Sainaghi *et al* demonstrated the relation between

low levels of vitamin D in different rheumatic diseases with higher production of PTH <sup>(37)</sup>. Recent Turkish study bone mass and bone turnover measurement for premenopausal women with FMS and degenerative disc disease control patients; the authors demonstrated that presence of depression, general pain and other clinical findings in FMS created women to be susceptible to osteoporosis <sup>(38)</sup>.

Oxidative stress (OS) and Co Q play a role in neuro- musculoskeletal conditions such as FM so the providing of antioxidants kept tissues faraway from unpleasant effects of OS <sup>(39-42)</sup>. In Egyptian study by Soliman *et al* showed that the level of (OS) increased with decreased of antioxidant capacity significantly these findings in concomitant with the present study in premenopausal age <sup>(43)</sup>. Another Spanish study determined total anti-oxidant capacity in 82 postmenopausal FM women and compared with 25 apparent healthy as control their results showed significantly decreased TAS when compared with controls( Zinc, Uric acid, Ferritin, iron, Catalase, SOD, GPx ) as a result of enhancement of OS in FM because of elevation of protein peroxidation and oxidative DNA damage significantly and generated advanced glycation end-products (AGEs) so this modified proteins became more resist to be digest, as well as excite cytokines, adhesion molecules, and growth factors expression through NF-  $\kappa$ B stimulant<sup>(44)</sup>. Niklowitz *et al* mentioned the blood level of Co Q was decreased in old German people so the redox status be shift to oxidized direction <sup>(45)</sup>.

The impact of obesity on current FMS study and comparison between obese and non-obese, looked through elevation of PTH increased significantly at the same time decreased of TAS serum level. This syndrome is one of musculoskeletal disorder with obesity agree with old study by Christensen *et al* in their demonstrating the combination of obesity with certain rheumatologic conditions specifically knee osteoarthritis <sup>(46)</sup>. Other studies showed the effects of obesity on clinical examination of FMS by measuring tenderness, symptoms, quality of life <sup>(47, 48)</sup>. Okifuji *et al* assessed catecholamines, cortisol, C-reactive protein and interleukin-6 as neuroendocrine indices as well as measuring some clinical manifestation such as symptoms, treadmill testing and indices of sleep in thirty eight FMS patients <sup>(49)</sup>. There are different mechanism to explain the association of fibromyalgia and obesity in accordance to endocrine dysfunction; one of them is impairment of growth hormone and insulin growth factor-1 (IGF-1) secretion this finding illustrated in FM patients by Bennett *et al.* and in obese subjects by Maccario *et al.*<sup>(50-52)</sup>. Authors in 2008 published their results that demonstrate bone mineral density and serum level of osteocalcin and IGF-1 which decreased significantly and insignificant decrease calcium, phosphorus, vitamin D3 and PTH in premenopausal

FMS with mean BMI  $25.63 \pm 3.14$  kg/m<sup>2</sup> (16). In Spain; Aparicio *et al.* observed the effect of obesity and over weight on pain, fatigue, stiffness and physical functioning and play a role in progress of these FM symptoms (53). The association between pain of FM and obesity showed in old study due to central sensitization (54,55). Kim *et al* mentioned that the greater BMI had greater fibromyalgia-related symptoms through their study on wide range of BMI by classify the patients into four groups (non- obese, overweight, moderately obese and severely obese) (56). Jameel M. G. *et al* demonstrated the effect of obesity on serum total calcium, myeloperoxidase and vitamin D3 in Iraqi FMS patients in addition to fetuin –A and thyroid function test in two different publishing studies (57,58). In the longitudinal analyses demonstrate an association between weight loss of obese middle-aged patients with localized weight-bearing joints pain by studying C reactive protein, interleukins, tumor necrosis factor- $\alpha$  and interferon- $\gamma$ (59). Brazilian authors studied the effect of overweight and obesity on serum levels of some adipokines in FM middle aged women (60). Turkish study include 124 FMS women classified into normal BMI, overweight and obese to assess pain, tender point count, disease activity, anxiety and depression in ages between 18- 55 years (61). Cross sectional study in Turkey was done to evaluate the effects of obesity and over weight on FMS severity in relation to general health and psychological status (62).

Conclusion: The study clarify the influence of progressing age in general but chiefly after menopausal age when compared with age less than 45 years as well as obesity on serum level of PTH, Co Q and TAS in Iraqi FMS women through enhancing the oxidative stress and tissue damage as result directly or indirect accelerating pathophysiology, etiology and severity. Thus FMS women must recommended anti-oxidant supplements, vitamin D and minerals especially calcium, as well as advise them to improve lifestyle in order to decrease the undesirable effects of FMS.

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