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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 and TAS) (P value >0.05 and 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 الحيويه في مصل النساء قبل وبعد سن اليأس في متلازمة الم الليف العضلي وعلاقتها بالسمنه

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الخلاصة

وضعت الدراسة لتقييم مستوى هرمون الجار الدرقي ،الانزيم المشارك ف وحالة مضادات التأكسد الكليه في النساء العراقيات المصابات بمتلازمة الم الليف العضلي اولا ولبيان تاثيرالعمر ومؤشر كتلة الجسم عليهم. تمت الدراسة في شعبة امراض الروماتيزم والتأهيل الطبي التابعه لمستشفى مدينة الطب بسحب نموذج من دم المريضات البالغ عددهم(٥٩) اضافة الى (٣٠) نموذج من مجموعة السيطره. معدل العمر ±المنحرف القياسي للمريضات وغير المريضات هو ٤٢,٢٢ ±١٥,٣٤ و ٤٠,٧±١٨,٢٢ سنه على التوالي. تم تقسيم المرضى والاصحاء الى اربعة مجاميع حسب العمر وكتلة الجسم ,معدل اعمار المريضات والاصحاء قبل سن اليأس(٣٠,١٤±١٠,٥٨ و ٢٩,٧٤ ± ١٢,٢١) سنه وبعد سن اليأس (٥٣,٢±١٦,١٨ و ٤٧,٨±١٢,٥١) سنه 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 زياده معنويه في مستوى الهرمون الجار الدرقي بالمصل ونقصان معنوي للأنزيم ولامعنوي لمضادات الأكسده.بالنسبه للعمر عند مقارنة مجموعتي (المريضات مع السيطره). لوحظ زياده غير معنويه للهرمون و قله للأنزيم, نقصان معنوي لمضادات الأكسده عند المقارنه بين قبل و بعد سن اليأس. بينت النتائج هناك زياده معنويه عند النساء البدينات المصابات بالمتلازمه عند مقارنتها بمجموعة السيطره لكن الاختلاف غير ملموس في مجموعتي البدينات وغير البدينات. وأيضا سجلت حالة مضادات الأكسده قله معنوية في مجموعة البدينات فقط.

وضحت النتائج هناك علاقه معكوسه معنويه 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) بالأعتماد على مؤشر كتلة الجسم لاكثر من ٣٠. ايضا هناك علاقه معنويه حرجه (P value=0.05) في قياس الهرمون بين المجموعتين اعلاه بعد سن اليأس.

علاقة التباين بأختبار تي لما (قبل ٤٥ وبعد ٥٥) سنه بينت وجود علاقه معنويه لمضادات الاكسده للمريضات فقط. وجدت علاقه معكوسه بين الهرمون والانزيم للمريضات البدينات وغير البدينات اضافة الى وجود تباين واضح بالهرمون ومضادات الاكسده لكلا المجموعتين (P value=0.032 and P value= 0.0001)

الكلمات المفتاحيه: السمنه، سن اليأس، متلازمة ألتهاب الليف العضلي،الهرمون الجار الدرقي،حالو 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 [(1)]. 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 [(1, 2)].

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

Coenzyme Q (Co Q) or Q_{10} 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 [(5, 6)].

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 [(7-9)]. The definition of TAS is the sum total of endogenous and food derived antioxidants of the extra cellular fluid of an individual [(10)].

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 [(11)]. 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^2)} [(12)] (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) [(13-15)]. 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.

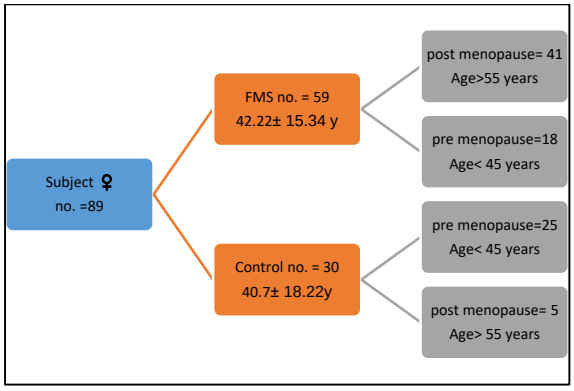

Figure1. Demographic distribution of volunteers (FMS and control) according to age in this study.

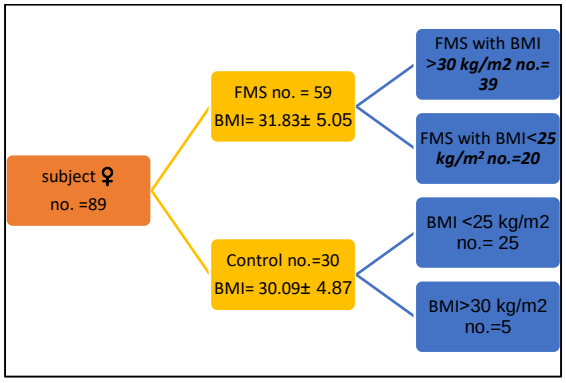

Figure2.Demographic distribution of (FMS and control) women according to BMI in this study.

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.

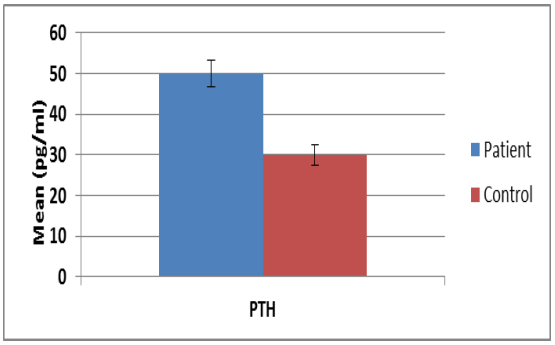

Figure 3. The mean serum PTH level (pg/ml) in FMS and control groups (P value<0.01)

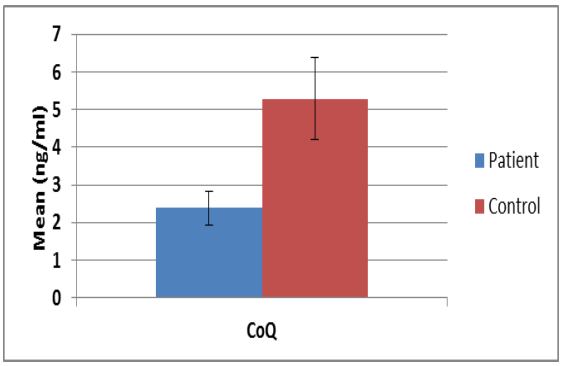

Figure 4. The mean serum Co Q level (ng/ml) in FMS and control (P value <0.01)

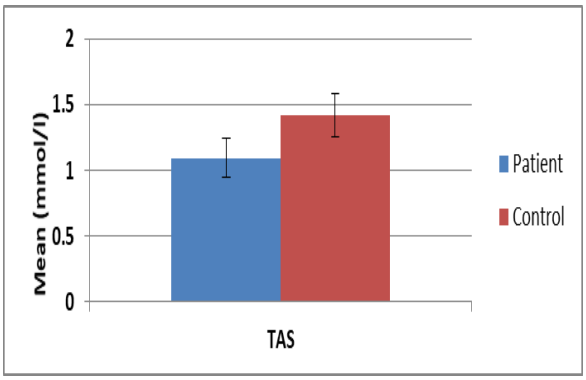

Figure 5.The mean serum TAS level (mmol/l) in both FMS and control groups (P value > 0.05)

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 [(16)].

The results show hyperparathyroidism of FMS group if comparing with control so it is in concomitant with the result of Armagan *et al* [17] 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 [18]. 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 (19). Previous studies [20, 21] show FMS have a predisposition to osteopenia. Measurement Co Q, in this study showed deceasing 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 [22]. Study on osteoarthritis demonstrated the same results [23]. In US [24]; 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 [25]. Chung e*t al* study match with current study in relation with oxidative stress and FMS [26].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 [27]. The role of oxidants/antioxidants, mitochondrial dysfunction, and autophagy in fibromyalgia was published recently [28].

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 [29] because an impairment of ovarian function in postmenopausal women alter the metabolism of Ca and therefore reduce bone mass [30]. Borgia *et al*. demonstrate the role of hyperparathyroidism in musculoskeletal pain of fibromyalgia [31]. In correspondence of present study with Cammozi *et al* and Safi *et al* observations that parathyroid hormone was significantly low in postmenopausal women [32,33]. 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 [34, 35]. The role of estrogen in inhibiting interleukin six was decreased with age so lead to prolongation of osteoclast's life span [36]. Sainaghi *et al* demonstrated the relation between low levels of vitamin D in different rheumatic diseases with higher production of PTH [37]. 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 [38].

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 [39-42]. 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 [43]. 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- κB stimulant[44]. 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 [45].

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 [46]. Other studies showed the effects of obesity on clinical examination of FMS by measuring tenderness, symptoms, quality of life [47, 48]. 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 [49].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*.[50-52]. 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± 3.14 kg/m2 [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-α and interferon-γ[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.

References

1. Anil Kumar Jain, Bruce M. Carruthers: Fibromyalgia Syndrome: A Clinical Case Definition and Guidelines for Medical Practitioners An Overview of the Canadian Consensus Document. Journal of Musculoskeletal Pain; 2003, 11(4):3107.
2. Wolfe F, Clauw DJ, Fitzcharles MA *et al*: The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and mea-surement of symptom severity. Arthritis Care Res; 2010, 62, 5; 600–610.
3. Roseann P. Velez, Mary Donnelly-Strozzo, and Julie Stanik-Hutt: Simplifying the Complexity of Primary Hyperparathyroidism. The Journal for Nurse Practitioners – JNP; 2016, 12, 5.
4. Megan K. Applewhite, David F. Schneider: Mild primary hyperparathyroidism: a literature review. Oncologist, 2014;19: 919-929
5. Bozkurt M, Oktayoglu P, Em S, Mehmet C *et al* : Serum Coenzyme Q10 Levels and Oxidative Status in Patients with Fibromyalgia Syndrome Journal Of Musculoskeletal Pain, Early Online: 1–6, 2014 Informa Healthcare USA, Inc. ISSN: 1058-2452 print / 1540-7012 online DOI: 10.3109/10582452.2014.883040
6. Quinzii CM, Lopez LC, Naini A *et al*: Human CoQ10 deficiencies. Biofactors, 2008; 32: 113–118.
7. Barry Halliwell and John M. C. Gutteridge: Free Radicals in Biology and Medicine Fifth Edition 2015 Oxford: Oxford Science Publication Chapter 10.
8. Young, I.S. and Woodside, J.V.: Antioxidants in Health and Disease. Journal of Clinical Pathology, 2001; 54, 176-186.
9. Sánchez-Domínguez B, Bullón P, Román-Malo L *et al*: Oxidative stress, mitochondrial dysfunction and, inflammation common events in skin of patients with Fibromyalgia; Mitochondrion, 2015, MITOCH-00992; No of Pages 7
10. Miller,N, Rice –Evans, C, Davies, MJ, A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci, 1993, 84, 407-412.
11. Koopman WJ., Boulware DW., Gustavo R. *et al*: Fibromyalgia in Clinical Primer of Rheumatology,14 th edition ,Lippincott Williams and Wilkins (editors) Philadelphia, 2003; 226-35.
12. World Health Organization (WHO): Obesity and overweight .*Fact sheet NO*.311, 2008.
13. Marcus, R.: Laboratory diagnosis of primary hyperparathyroidism, Endocrinol Metab. Clin. North Am., 1989; 18:647-658.
14. Frances T Fischbach, Marshall B Dunning: Immunodiagnostic Studies in A Manual of Laboratory and Diagnostic Tests; ninth ed.; 2014, Chapter 8. Wolters Kluwer health / Lippincott Williams & Wilkins.
15. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem., 2004; 37(4):277-85.
16. Eich W, Häuser W, Arnold B *et al*: Fibromyalgia syndrome. Definition, classification, clinical diagnosis and prognosis, Der Schmerz, 2012; 3; 1-10· Published by Springer
17. Armağan O, Sırmagül ε, Ekim A *et al* : Levels of IGF-1 and Their Relationship with Bone Mineral Density in the Premenopausal Women with Fibromyalgia Syndrome; Rheumatism, 2008; 23: 118-23

18. Shaheen A, Niaz A, Muhammad I *et al*: Vitamin D deficiency in Fibromyalgia. J Pak Med Assoc, 2010; 60 (11), 949-951.

19. Ferrari R& Science A: Prevalence of primary hyperparathyroidism in a referred sample of fibromyalgia patients; Clinical Rheumatology. 2015; 34 (7), 1279-1283.

20. Zerahn B, Bliddal H, Moller P *et al*.: Bone mass in the calcaneus in patients with fibromyalgia. J Musculoskeletal Pain, 2001; 9: 17-23.

21. Swezy RL& Adams J.: Fibromyalgia: a risk factor for osteoporosis. J Rheumatol 1999; 26: 2642-4.

22. Meeus M, Nijs J, Hermans L *et al.*: The role of mitochondrial dysfunctions due to oxidative and nitrosative stress in the chronic pain or chronic fatigue syndromes and fibromyalgia patients: peripheral and central mechanisms as therapeutic targets? Expert Opin Ther Targets. 2013; 17(9):1081-9.

23. Altındağ Ö, Koçyiğit A, Çelik N *et al*: DNA Damage and Oxidative Stress in Patients with Osteoarthritis: a Pilot Study; Rheumatism 2007; 22: 60-3.

24. National Cancer Institute (NCI). "PDQ® Coenzyme Q10". NCI, National Institutes of Health, US Dept. of Health and Human Services. "Thiamine, thiamine phosphates and their metabolizing enzymes in human brain" J Am Chem Soc. 2014; 80 (14): 37193726.

25. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet. 1994; 344(8924):721-4.

26. Chung C, Titova D, Oeser A, *et al*: Oxidative Stress in Fibromyalgia and its Relationship to Symptoms: Clin Rheumatol. 2009; 28(4): 435–438.

27. Yildirim T and ALP R: The role of oxidative stress in the relation between fibromyalgia and obstructive sleep apnea syndrome; European Review for Medical and Pharmacological Sciences: 2017; 21: 20-29

28. Alejandra M& Simón R: The Role of Oxidants/Antioxidants, Mitochondrial Dysfunction, and Autophagy in Fibromyalgia; INTECH; 2018, Chapter 2, 13-33.

29. Guncha K and Gagan D: Serum Calcium, Parathyroid Hormones and Risk of Fracture in Premenopausal and Postmenopausal Women; Annals of International Medical and Dental Research, 2017; 3(5): OR24-OR27.

30. Garton M, Martin J, New S, *et al*: Bone mass and metabolism in women aged 45–55. Clin Endocrinal (Oxf). 1996; 44:563–73.

31. Borgia AR, Cavallasca JA, Costa CA *et al.*: Hyperparathyroidism: A Forgotten Cause of Musculoskeletal Pain; Rheumatol Clin. 2012; 8(5):298–301

32. Cammozi V, Lumachi F, Mantero F, *et al*: Phalangeal quantitative ultrasound technology and dual energy X-ray densitometry in patients with primary hyper parathyroidism: influence of sex and menopausal status. Osteoporosis International; 2003, 14 (7), 602-608

33. Safi S, Hassikou H, Messary A, *et al*: Severe primary hyperparathyroidism and vitamin D deficiency. Ann Endocrinol (Paris) 2004; 65(3):226-32

34. Eastell R, Yergey AL, Vieira N, *et al.*: Interrelationship among vitamin D metabolism, true calcium absorption, parathyroid function and age in women: evidence of an age-related intestinal resistance to 1, 25 $(OH)_2$ D action. J Bone Miner Res 1991; 6:125–32.

35. Khosla S, Elizabeth J, Atkinson L, *et al.*: Effects of age and estrogen status on serum parathyroid hormone levels and biochemical markers of bone turnover in women: A population based study. J Clin Endocrinal Metabol. 1997; 82: 1522–7.

36. Garneo P& Delmas PD. Bone turnover markers. In: Encyclopedia of Endocrine Diseases. Eds Martin L. California: Elsevier Inc; 2004.pp.401–13.

37. Sainaghi PP, Bellan M, Antonini G et al.: Unsuppressed parathyroid hormone in patients with autoimmune/inflammatory rheumatic diseases: implications for vitamin D supplementation. Rheumatology (Oxford). 2011 Dec; 50(12):2290-6.

38. Gurbuz A, Kaya A and Ansal T: Bone mass and bone turnover in premenopausal women with fibromyalgia syndrome; Medicine Science International Medical Journal; 2017, 06; 8669.

39. Miyamae T, Seki M, Naga T, *et al.*: Increased oxidative stress and coenzyme Q10 deficiency in juvenile fibromyalgia: amelioration of hypercholesterolemia and fatigue by ubiquinol-10 supplementation. Redox Rep. 2013; 18: 12-19.

40. Fatima G, Das SK, Mahdi AA: Oxidative stress and anti-oxidative parameters and metal ion content in patients with fibromyalgia syndrome: implications in the pathogenesis of the disease. Clin Exp Rheumatol. 2013; 31: 128-133.

41. Cordero MD, Alcocer-Gómez E, de Miguel M, *et al.* Can coenzyme q10 improve clinical and molecular parameters in fibromyalgia? Antioxid Redox Signal. 2013; 19: 1356-61.

42. Pereira AP, Pereira LL, de Souza LC, *et al*: Involvement of Oxidative Stress and Nitric Oxide in Fibromyalgia Pathophysiology: A Relationship to be Elucidated. Fibrom Open Access 2016, 1:1

43. Soliman AF, El-Olemy GG, Hassan WA, *et al*: Impact of an intensive dynamic exercise program on oxidative stress and on the outcome in patients with fibromyalgia .Egyptian Rheumatology & Rehabilitation 2016, 43 (3); 117-123

44. Rubia M La, Rus A, Molina F, *et al*: Is fibromyalgia-related oxidative stress implicated in the decline of physical and mental health status? *Clin Exp Rheumatol* 2013; 31 (79): S121-S127.
45. Niklowitz P, Onur S, Fischer A, *et al:* Coenzyme Q10 serum concentration and redox status in European adults: influence of age, sex, and lipoprotein concentration J Clin Biochem Nutr. 2016; 58(3): 240–245
46. Christensen R, Astrup A, Bliddal H: Weight loss: the treatment of choice for knee osteoarthritis? A randomized trial. Osteoarthritis Cart. 2005; 13:20–27.
47. Bennett RM, Jones J, Turk DC, *et al*: An internet survey of 2, 596 people with fibromyalgia. BMC Musculoskelet Disord. 2007; 8:27.
48. Neumann L, Lerner E, Glazer Y, *et al*: A cross-sectional study of the relationship between body mass index and clinical characteristics, tenderness measures, quality of life, and physical functioning in fibromyalgia patients. Clin Rheumatol. 2008; 27(12): 1543–1547.
49. Okifuji A, Bradshaw DH, Olson C: Evaluating obesity in fibromyalgia: neuro endocrine biomarkers, symptoms, and functions. Clin Rheumatol. 2009; 28(4):475–478.
50. Bennett RM, Clark SR, Campbell SM *et al*: Low levels of somatomedin C in patients with the fibromyalgia syndrome. A possible link between sleep and muscle pain. Arthritis Rheum. 1992; 35(10):1113-1116.
51. Bennett RM, Cook DM, Clark SR *et al*: Hypothalamic-pituitary-insulin-like growth factor-I axis dysfunction in patients with fibromyalgia. J Rheumatol 1997; 24(7):1384–1389
52. Maccario M, Grottoli S, Procopio M *et al* The GH/IGF-I axis in obesity: influence of neuro-endocrine and metabolic factors. Int J Obes Relat Metab Disord. 2000; 24(S2):S96–S99
53. Virginia A. Aparicioa, Francisco B. Ortega, Ana Carbonell-Baezaa *et al*: Relationship of Weight Status with Mental and Physical Health in Female Fibromyalgia Patients, *Obes Facts.* 2011; 4: 443–448
54. Okifuji A, Donaldson GW, Barck L *et al*: Relationship between fibromyalgia and obesity in pain, function, mood, and sleep. *J Pain.* 2010; 11(12):1329-1337.
55. Yvonne D'Arcy: Managing pain in obese patients; The Nurse Practitioner, 2011; 36 (12); 28-32
56. KIM CH, LUEDTKE CA, VINCENT A *et al*: Association of Body Mass Index With Symptom Severity and Quality of Life in Patients With Fibromyalgia; Arthritis Care & Research, 2012; 64 (2), 222–228
57. Murooj G J, Eman S S, Mohammad H A: Vitamin D and oxidative stress in obese Iraqi sample with fibromyalgia syndrome. Int. J. Res. Dev. Pharm. L. Sci, 2015, 5(1), 1974-1980
58. Murooj G J, Eman S. S: Evaluation of fetuin A in obese and non- obese Iraqi women with fibromyalgia syndrome; Bulletin of Pharmaceutical and Medical Sciences (BOPAMS); 2017, 5 (1), 5001-5006
59. Andrew S, Steven E, Nicole M *et al*: Improvement in the Spatial Distribution of Pain, Somatic Symptoms, and Depression after a Weight Loss Intervention; The Journal of Pain, 2017; 18 (12): 1542-1550
60. Eduardo S, Aline A, Emmanuelle D *et al:* Serum levels of leptin and adiponectin and clinical parameters in women with fibromyalgia and overweight /obesity, Arch Endocrinol Metab. 2017;61/3;249-256
61. Koc¸yig˘it and Okyay: The relationship between body mass index and pain, disease activity, depression and anxiety in women with fibromyalgia. PeerJ; 2018, 6:e4917;1-10 DOI 10.7717 / peerj.4917
62. Mehmet O Ç, Burcu D Ç, Hakan GS *et al*: The Association of Skinfold Anthropometric Measures, Body Composition and Disease Severity in Obese and Non-obese Fibromyalgia Patients: A Cross-sectional Study; Arch Rheumatol 2018;33(1):59-65.