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Research Article

VITAMIN D AND OXIDATIVE STRESS IN OBESE IRAQI SAMPLE WITH FIBROMYALGIA SYNDROME

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ABSTRACT

Fibromyalgia syndrome(FMS) is chronic state characterized by generalized pain associated with fatigue, stiffness, altered sleep, depression, anxiety and cognitive dysfunction. It is the most common condition in women worldwide and causes pain all over the body . The exact cause of FMS is still unknown but several theories thought to be linked to abnormal levels of certain chemicals in brain ,vitamin D deficiency and oxidative stress . Obesity has been consistently associated with increased risk of FMS. Eighty nine subjects between (20-55)years ; (59) patients with FMS were divided in to : (39) obese women with FMS and (20) non obese women with FMS . In addition to two controls group ;(20) obese control and (10) apparently healthy control, age and sex matched subjects as controls. Five millilitres of venous blood sample were drawn from each women ,centrifuged to obtain serum to be used for measuring the following variables :25(OH)vitamin D, total calcium and myeloperoxidase. 25(OH)vitamin D and total calcium were significantly lower, while myeloperoxidase was significantly higher in FMS with obesity and non-obesity . Body mass index (BMI) is significantly higher in FMS with obesity and non-significant in FMS with non-obesity. A low 25(OH) vitamin D level was positively associated with low total calcium level in FMS with obesity and non-obesity . A high myeloperoxidase was associated with low levels of 25(OH) vitamin D and total calcium in FMS with obesity . The Lower serum levels of 25(OH)vitamin D , serum total calcium and higher serum myeloperoxidase and higher BMI are associated with FMS with obesity.
Keywords: Fibromyalgia syndrome, 25(OH) vitamin D, total calcium oxidative stress, myeloperoxidase.

INTRODUCTION

Fibromyalgia syndrome (FMS) is chronic state characterized by generalized pain associated with fatigue, stiffness, altered sleep , depression , anxiety and cognitive dysfunction(Smith et al., 2011). Fibromyalgia syndrome is a second common rheumatic disorder , it may to be affect 2-8% of the general population (Vincent et al., 2013) . 25(OH) vitamin D, total calcium and myeloperoxidase that believed to have a key position in the pathogenesis of FMS. Vitamin D is considered as a steroid hormone acts on the musculoskeletal system which associated with symptoms linked to its deficiency could be responsible for widespread muscle pain and also in FMS (Altindag et al., 2014). Vitamin D refer to group of fat soluble secosteroid ,that important for enhancing intestinal absorption calcium, magnesum, zinc and phosphate , with two majors biologically inert vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Both vitamins D2 and D3 obtained from diet and supplements ; vitamin D3 is also synthesized in the skin via exposure the 7- dehydrocholesterol to the sun light (Holick, 2006, 2007). Vitamin D have a role in adaptive immunity with vitamin D receptor (VDR) . It's activating enzymes express in both T and B cells. The presence of T

cells with vitamin D inhibit the secretion of pro – inflammatory Th-1 cytokines such as TNF - α and 1L – 1(Holló et al., 2012). Calcium is considered as essential mineral elements in the body. It is accounts 2% of body weight in adults. About 99% of Ca^{+2} is present in the skeleton and teeth and the remainder about 1% of total body calcium is found in the soft tissues and body fluids (Pravina et al., 2013). Calcium play an important role in nerve transmission , muscle contraction and relaxation (Ilich and Kerstetter, 2000). Oxidatvie stress have an important role in the pathophsiology of FMS (51). Myeloperoxidase (MPO) is a human peroxidase enzyme and lysosomal protein that stored in azurophilic granules of the neutrophil . Myeloperoxidase have molecular weight 114 kilo Dalton (kDa), consists of two identical monomers 72 kDa that linked by disulphide bridge , each monomer consists of a light and heavy chain (Fiedler et al., 2000). Myeloperoxidase play a crucial role in the inflammation and the oxidative stress at cellular level (Anatoliotakis et al., 2013).

MATERIALS AND METHODS

The study was carried out over 6 months period from September 2014 till January 2015 at Medical City – Baghdad Teaching Hospital - Rheumatolog and Rehabilitation Consultation Unit . This study consists of two patient groups ,the first group was composed of 39 obese women with FMS and the second group composed of 20 non obese women . In addition to two control groups ; 20 apparently healthy women as control group and 10 obese women as obese control , both matched for BMI of fibromyalgia syndrome patients . The fibromyalgia syndrome (FMS) was diagnosed by American College of Rheumatology (ACR) criteria (Koopman et al., 2003). This study was approved by Clinical Research Ethics Committee of Pharmacy College University of Baghdad Five milliliters (5ml) of Venous blood sample were drawn from each subject (patients and control).The sample placed in gel-containing tubes ,left at room temperature for at least 30 minutes for clotting, then centrifuged at 1000 round per minute (rpm) for 10 minutes in ordered to obtain serum, then separated and divided into aliquots to measure vitamin D, total calcium and myeloperoxidase .The serum kept frozen at -20°C until analysis used to measure vitamin D and myeloperoxidase. Measurement of 25(OH)vitamin D and myeloperoxidase levels were performed by Enzyme – Linked Immune Sorbent Assay (ELISA), using a commercially available Kits, human 25(OH) vitamin D ELISA kit and human myeloperoxidase ELISA kit . The principle of this technique is based on a quantitative Sandwich –Assay by using two specific and high affinity antibodies, the plate of microtiter has been pre coated with an antibody specific to the substance to measure .The results were expressed as nanogram per milliliter (Klebanoff, 1999; Snellman et al., 2010), while total calcium was measured by colorimetric method. The principle of the method is based on the formation of a purple colored complex, when the calcium ions (Ca+2) react with o-cresolphthalein-complexone in an alkalaine medium . The values were expressed in mg\dl (Philip, 1994).

Statistical Analysis

All data analyzed using Minitab version 17 computer program. Statistical Analysis involved descriptive statistics, tables and figures . Statistical Analysis also included t-test and Person correlation coefficient test for quantitative variables. In this analysis, Person correlation coefficient was determined , p values were based on 2- sided tests and p value less than 0.05 was considered statistically significant.

RESULTS

Baseline Demographic and Clinical Characteristic

The clinical characteristic and baseline demographic of the study groups, as well as Biochemical parameters of the patients and controls as shown in table 1 and table 2 .The study group included 89 subjects 59 patients (39 FMS with obesity and 20 FMS with non-obesity) and controls (20 obese control and 10 apparently healthy control) . As shown in table 1,the mean age for patients (FMS with obesity and FMS with non-obesity) and controls (obese and apparently healthy controls) were 42.49±8.36 ; 30.15±5.98 ; 36.55 ± 9.10 ; 34.2±6.01 years respectively . The mean value of age for FMS with obesity was significantly higher than obese control ,while mean value of age was non-significant between FMS with non-obesity than healthy control . The mean value of BMI for patients and controls were 32.55±3.99 ; 22.94±1.59 ;

Table 1 : Baseline Demographic and Clinical Characteristic of Women Enrolled in the Study

	Fibromyalgia syndrome (FMS) with obesity	Obese control	Fibromyalgia syndrome with non obese	Healthy control
Number of subjects	39	20	20	10
Age (year)	42.49±8.36*	36.55 ± 9.10	30.15±5.98	34.2±6.01
BMI (kg\m²)	32.55±3.99 *	29.78±3.83	22.94±1.59	22.98±1.75
Social condition	94%	75%	80%	90%
Married Single	5%	25%	20%	10%
Occupation%				
House wife	89%	80%	85%	80%
Working	10%	20%	15%	20%

P value < 0.05 considered significant

Table 2: Mean ±SD for biochemical parameters of the patients and controls.

	Fibromyalgia syndrome with obesity	Obese control	P value*	Fibromyalgia syndrome with non obesity	Healthy control	P value*
25(OH)Vitamin D (ng\ml)	5.22 ± 3.59	27.83 ±6.06	< 0.001	6.97 ±4.91	27.74 ±1.71	<0.01
Total Calcium (mg\dl)	8.30±1.31	9.36±1.26	0.001	7.90±1.04	9.78±0.752	< 0.001
Myeloperoxidase (ng\ml)	97.6±41.9	31.6±27.9	< 0.001	109.1±63.8	29.1±17.6	< 0.001

P value < 0.05 considered significant

Table 3: Correlation Coefficient and P values of 25(OH) Vitamin D, Total Calcium and Myeloperoxidase in FMS patients with obesity

Variable		Total Calcium	Myeloperoxidase
Serum 25(OH) Vitamin D	R value	0.465	-0.422
	P value	0.003**	0.007**
Serum Total Calcium	R value		-0.371
	P value		0.02*

(*) Correlation is significant at the 0.05 level (2 –tailed).

(**)Correlation is significant at the 0.01 level (2-tailed)

Table 4: Correlation Coefficient and P values of 25(OH) Vitamin D , Total Calcium and Myeloperoxidase in FMS patients with non-obesity.

Variable		Total Calcium	Myeloperoxidase
Serum 25(OH) Vitamin D	R value	**0.663**	**-0.492**
	P value	**0.001****	**0.027***

(*) Correlation is significant at the 0.05 level (2 –tailed)

(**) Correlation is significant at the 0.01 level (2-tailed)

29.78±3.83 ; 22.98±1.75 Kg/m^2 respectively ,the BMI of FMS patients with obesity was significantly higher than the BMI of obese control ($p < 0.05$) ,while non-significant between FMS patients with non-obesity and healthy control ($p > 0.05$) . Table 2 show the Mean ±SD for serum levels of 25(OH) Vitamin D , total calcium and Myeloperoxidase in controls and patients . Fibromyalgia syndrome patients with obesity showed lower 25(OH) vitamin D and total calcium than obese controls ($p < 0.001$),($p= 0.001$) respectively . Fibromyalgia syndrome patients with non-obesity also showed lower levels of 25(OH) vitamin D and total calcium than healthy control ($p < 0.01$),($p < 0.001$) respectively . Myeloperoxidase level was significantly higher in FMS patients with obesity and non-obesity than obese control and healthy control ($p < 0.001$),($p < 0.001$) respectively .

Correlations Studies:

Correlation Coefficient and P values of 25(OH) Vitamin D , Total Calcium and Myeloperoxidase among all studied groups combined .

As shown in table 3 the serum 25(OH) vitamin D level was positively correlated with total calcium level ($p <0.01$) ,this was in contrast to myeloperoxidase which correlated negatively with serum 25(OH) vitamin D and total calcium ($p <0.01$) . Table 4, also show the serum 25(OH) vitamin D level was positively correlated with total calcium level ($p <0.01$) ,this was in contrast to myeloperoxidase which correlated negatively with serum 25(OH) vitamin D ($p < 0.05$) .

DISCUSSION

Fibromyalgia syndrome (FMS) is a complex problem in which low levels of 25(OH) vitamin have been reported (Armstrong et al., 2007) .In this study serum 25(OH)vitamin D levels was significantly lower in obese and non-obese women with FMS than obese control and healthy controls respectively ,this was in accordance with several studies (Heidari et al., 2010; Olama et al., 2013). Vitamin D play a role in FMS , There are several mechanism on how vitamin D affects the pain process, vitamin D can modulate neuronal excitability as similar to other neuroactive steriod (Mensah-Nyagan et al., 2009). This involves spontaneous regular firing, intrinsic excitability, also action potential duration and sensitivity to the neurotransmitters and neurotransmitter receptors (Feldman et al., 2007). Vitamin D also modulate the brain neurotransmitters such as dopamine and serotonin (Mensah-Nyagan et al., 2009).Vitamin D is affects the inflammatory pathways that associated with development of chronic pain, its upregulate the transforming growth factor beta 1 (TGF-B1) and interleukin -4 that found in asrocytes and microglia(Marchand et al., 2005). Transforming growth factor beta 1 suppress the activity of cytokines such as interferon- α , tumor necrosis factor –alpha and interleukin-1(1L-1). It can also downregulate the activity of immune cells .Vitamin D suppresses the tumor necrosis factor –alpha (TNF- α) and macrophage stimulating factor (M-CSF) in astrocyte and microglia (Marchand et al., 2005), M-CSF is define as a cytokine that stimulates proliferation , differentiation and also survival of monocytes and the macrophages . Macrophages release many inflammatory mediators such as proinflammatory cytokines , nitric oxide (NO) and neural growth factor (NGF) (Bilal et al., 2009). In the present study serum total calcium level was significantly lower in obese and non-obese women with FMS than obese control and healthy controls respectively. This was as the same in Magaldi et al., (2000) study who found that serum calcium was decreased significantly in FMS cases compared to controls. Calcium decrease oxidative stress marker, malondialdehyde and increase antioxidant enzymes such as peroxidase and superoxide dismutase (SOD) (Yew and DeMieri, 2002). Positive significant correlation was found in FMS with obesity and non-obesity between 25(OH) vitamin D and total calcium .This was consistent with the finding of other study that reported vitamin D was positively correlated with serum calcium , indicating that vitamin D is included in absorption of calcium (Heaney, 2003; Heaney et al., 2003; Kiran et al., 2014) . In this study, serum myeloperoxidase levels was significantly higher in obese and non-obese women with FMS than obese control and healthy controls respectively, this show the presence of the oxidative stress in the patients. In line with other studies investigating oxidative stress marker in fibromyalgia syndrome (Bagis et al., 2005; Ozgocmen et al., 2006). Myeloperoxidase (MPO) is a heme enzyme whose

expression is presented in both brain and immune cells , which indicate its important role in regulation of inflammatory processes and also formation of the oxidaive stress (OS) (Jameson et al., 2008). It's also induce lipid peroxidation (Hansson et al., 2006) . Oxidative stress theroy can play a crucial role in FMS pathophsiology ; however ,it still not clear whether OS abnormalities in the fibromyalgia syndrome are cause or the consequence (Jeschonneck et al., 2000). One possible mechanism explaining association between oxidative stress and FMS in obese patients is the presence of excessive adipocity because adipocytes and preadipocytes have been found as a source of inflammatory cytokines (Cordero et al., 2010). Cytokines are potent stimuli for production of reactive oxygen species and reactive nitrogen species by the macrophages and monocytes . Specifically cytokines increase the activity of oxidant generating enzymes myeloperoxidase (Dandona et al., 2001). This enzyme generating ROS and induce lipid peroxidation (Van der Veen et al., 2009). Lipid peroxidation of the cell membranes can modify the receptors accessibility, ligand binding and action, therefore altering the neurotransmitter functions (Lenaz, 1987). Ozogocmen et al., (2006) indicating that ROS induced muscle and neuron damages have an important role in pathophsiology of the muscular disorder; although, there are various antioxidant mechanism in muscles that neutralize the harmful effects of the ROS, tissue hypoxia predispose to loss of efficacy of antioxidant mechanism that may be accompanied with formation of the free radical. The other mechanism syndrome is influence of the local hypoxia due to disturbed microcirculation and leading to vasoconstriction in the skin of the tender point , hypoxia may result in ROS production and reduced antioxidant levels , which may contribute to sign and symptoms of fibromyalgia syndrome linked to the oxidative stress (Jeschonneck et al., 2000). In this study vitamin D and myeloperoxidase are negatively associated in FMS with obesity and non-obesity, this result is supported by Codoñer-Franch et al., (2012), while no available data about a correlation in non-obese FMS patients. In this study a negative correlation between serum total calcium and serum myeloperoxidase levels was also found in FMS patients with obesity . Bing H et al study the effect of calcium on antioxidant activities and who found that calcium could increase the activities of the antioxidant enzymes and decrease the lipid peroxidation (Bing et al., 2010).

CONCLUSION

1. Serum levels of 25(OH) vitamin D and total calcium is significantly decreased and serum myeloperoxidase is significantly increased in patients with fibromyalgia syndrome (FMS) with obesity and non obesity in a sample of Iraqi women .
2. Body mass index is significantly higher in FMS patients with obesity ,while non significant in FMS patients with non obesity
3. In future 25(OH) vitamin D and myeloperoxidase levels may be used to evaluate patients with suspected FMS.

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