



## Oxidative stress with relation to osteoporosis in married and unmarried menopause women in Kurdistan, Iraq

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### Abstract

To determine the differences in oxidative stress serum level between married and unmarried (maiden) in menopausal women with osteoporosis. During the period from May 8<sup>th</sup> 2014 till January 15<sup>th</sup> 2015. A total of 141 samples were collected, divided into three groups the first group represent (osteoporosis and osteopenia) all menopause, the second group represent (menopause) group and the third group represent (control) group. The patient has been classified according to age, educational level, residency, smoking and BMI. This study showed significant variation in GSR, MDA serum level were detected between low BMD group and control group, also between married and unmarried (maiden) group show significant difference, especially with maiden group. The results indicated an increase in oxidative stress serum level in unmarried menopause women especially maiden.

Keywords: Osteoporosis, Oxidative stress, Married women, Unmarried women.

### Introduction

Osteoporosis is a degenerative bone disease characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility (Wehrli *et al.*, 2006). Oxidative stress, resulting from excessive generation of reactive oxygen species, can damage all components of the cell (Sharma and Bhushan, 2012). At present, numerous studies have shown the positive correlation between oxidative stress and osteoporotic status (Zhang *et al.*, 2013). Oxidative stress can be defined as an excessive amount of ROS (reactive oxygen species) (Uttara *et al.*, 2009), which is the net result of an imbalance between production and destruction of ROS (Poljsak *et al.*, 2013). Oxidative stress is a consequence of an increased generation of free radicals and/or reduced physiological activity of antioxidant defenses against free radicals (Khalid *et al.*, 2007). Oxidative stress has been defined as harmful because oxygen free radicals attack biological molecules such as lipids, proteins, and DNA (Lobo *et al.*, 2007). However, more accurate definition of oxidative stress may be a state where oxidative forces exceed the antioxidant systems due to loss of the balance between them. The biomarkers that can be used to assess oxidative stress *In vivo* have been attracting interest because the accurate

measurement of such stress is necessary for investigation of its role in lifestyle diseases as well as to evaluate the efficacy of treatment (Yoshikawa and Naito, 2002).

Menopause has been reported to be associated with increased oxidative stress and metabolic disorders among women worldwide. Disarrangements in the redox state similar to those observed in women during the decline of ovarian hormonal activity can be obtained experimentally through rat bilateral oophorectomy (Behr *et al.*, 2012). Recently, oxidative stress has been reported as participating in the development of osteoporosis (Martha and Sanchez, 2007).

In women, the loss of bone occurs at a faster rate after the menopause, attesting to the adverse role of estrogen deficiency on bone mass and its contribution to the acceleration of skeletal involution with age (Maria *et al.*, 2013). Estrogen deficiency causes an increase in bone remodeling, increased osteoclast genesis and osteoblast genesis, increased osteoclast and osteoblast numbers, and increased resorption and formation-albeit unbalanced. Conversely, estrogens slow the rate of bone remodeling and promote a positive balance between bone formation and resorption by attenuating the generation of osteoclast and osteoblast progenitors in the bone marrow and

exerting a proapoptotic effect on osteoclasts and an antiapoptotic effect on osteoblasts and osteocytes (Maria *et al.*, 2013; Stavros C. *et al.*, 2013).

The Objective of the study to determine the difference in oxidative stress serum level between married and unmarried (maiden) in menopausal women with osteoporosis.

### Materials and Methods

This study was conducted in DEXA scan Department at Shahid Hemn Hospital in Sulaimania city, during the period from 8 May 2014 till 15 January 2015. This study included 141 patients. A total of 141 samples (married, unmarried) were included in this study. Among these, 117 were osteoporosis (married and unmarried) who were chosen from Sulaimani DEXA scan Department at Shahid Hemn Hospital in Suleiman. Their ages between 40 to 65 years. The remainders were 40 aged between 35 to 65 normal without osteoporosis and 45 apparently healthy subjects (control group) of comparable ages (30-47 years) who were not complaining of osteoporosis, used as a control group. A total of 141 patients during eight months of the study were interviewed and informed about the nature of the study and then asked to take part in the study.

This study the patient classified into three groups according to the DEXA scan was based on the clinical diagnosis as following:

- Osteoporosis patients, all menapauses (Low BMD), (married and unmarried) .
- Menopause (Married and unmarried)
- Control group (Young age premenopausal).

**Statistical Analysis:** This study was conducted using in depended-sample T-test, paired-sample T-test, chi-square and descriptive statistics such as (frequency, percentage, mean, standard deviation, standard error and histogram). Because the study was intendant to investigate oxidative stress in married and unmarried osteoporosis patient. The respondents in this study were under the number of patient and also the sample consist of 141 samples selected from all patients of Dexa scan center at sulaimania city, however only 117 questioners were correctly filled. Data from questioners were compiled, sorted, edited, classified and coded into a coding sheet in excel and analyzed using a computerized data analysis package known as statistical package for social science 17.0.

### Results and Discussion

**Patient distribution:** This study include the investigation of a total 141 patient, 61 (43.3%) were osteoporosis, 56 (39.7%) were osteopenia and 24 (17%) were menopause.

Table (1): Percentage distribution of patients

Class	Frequency	Percentage
Osteoporosis with menopause	61	43.3%
Osteopenia with menopause	56	39.7%
Menopause	24	17%
Total	141	100.0

**Unmarried Distributions:** The (117) low BMD patient (49) unmarried subdivided to three groups, (Table 2).

Table (2): The unmarried groups of low BMD

Class	Frequency	Percentage
Widow	17	34.7%
Divorced	8	16.3%
Maiden	24	49.0%
Total	49	100.0

Serum level of both GSR and MDA in married, unmarried groups of low BMD and menopause: The results obtained revealed a significant different in GSR, MDA between married and unmarried groups as shown in Table (3).

The GSR, MDA serum level difference between married and maiden patients: The results obtained revealed a significant higher in maiden group. The ( $P < 0.05$ ) of GSR, MDA in low BMD group, as a result it is statistically significant of GSR, MDA between married and un married.

The ( $P < 0.01$ ) for GSR, MDA of low BMD group, as a result it is fully statistically significant between married and maiden group. Whereas in menopause the ( $P > 0.05$ ) for GSR, MDA so it is not statistically significant between married and maiden in menopause group.

Serum level of ALP, Ca, Mg, Vit. D and PTH in Married and Unmarried Patients: The ( $P < 0.01$ ) of ALP and Calcium in low BMD it is fully statistical significant between married and unmarried. The ( $P > 0.05$ ) of Mg Vit. D and PTH in low BMD ,it is not statistically significant between married and un married. In menopause group the ( $P > 0.05$ ) thus it is not statistically significant between married and unmarried of ALP, Ca, Mg, Vit. D, and PTH.

Serum level of ALP, Ca, Mg and vit. D in married and maiden patients: Serum level of ALP, Ca and Mg in maiden group significantly lower than that of married osteoporotic patient ( $P < 0.05$ ), while no significantly different between maiden and married group in Vit. D ,PTH serum level ( $P > 0.05$ ).

Table (3): Comparison of mean  $\pm$ S.E. of GSR and MDA serum level between married and unmarried

			Low BMD	Menopause	Control
Total glutation (GSR) $\mu$ mol/L	married	Mean	5.97	5.87	4.1
		SD	0.314	0.24	0.38
		SE	$\pm$ 0.037	$\pm$ 0.24	$\pm$ 0.38
		n	68	18	45
	unmarried	Mean	6.09	5.9	-
		SD	0.314	0.29	-
		SE	$\pm$ 0.44	$\pm$ 0.12	-
		N	68	6	-
Malondi- aldehyde (MDA) nmol/L	married	Mean	2.64	2.33	1.23
		SD	0.183	0.169	0.38
		SE	$\pm$ 0.022	$\pm$ 0.03	$\pm$ 0.05
		N	68	18	-
	Unmarried	Mean	2.76	2.34	-
		SD	0.415	0.135	-
		SE	$\pm$ 0.059	$\pm$ 0.05	-
		N	49	6	-

Table (4): Comparison of mean $\pm$ S.E. of GSR, and MDA serum level between married and maiden of each low BMD, and menopause

			Low BMD	Menopause	Control
Total glutation (GSR) $\mu$ mol/L	married	Mean	5.97	5.87	4.1
		SD	0.312	0.24	0.38
		SE	$\pm$ 0.037	$\pm$ 0.05	$\pm$ 0.05
		N	68	18	45
	maiden	Mean	6.25	5.9	-
		SD	0.28	0.29	-
		SE	$\pm$ 0.057	$\pm$ 0.12	-
		N	24	6	-
Malondi- aldehyde (MDA) nmol/L	married	Mean	2.64	2.33	1.23
		SD	0.18	0.169	0.38
		SE	$\pm$ 0.25	$\pm$ 0.03	$\pm$ 0.05
		N	68	18	45
	maiden	Mean	2.92	2.34	-
		SD	0.466	0.135	-
		SE	$\pm$ 0.095	$\pm$ 0.05	-
		N	24	6	-

The data obtained were compared with the results of 45 control patients. The results reflect a remarkable increase in GSR and MDA is shown in unmarried osteoporosis and osteopenia patients, than GSR and MDA in married group as in (Table 3). Among the osteoporosis patients, specifically unmarried groups, the unmarried patients (maiden) showed an increase in GSR and MDA level as shown in (Table 4). Oxidative stress can lead to oxidative damage that affects all of the cellular components,

including proteins, lipids and nucleic acids (Bayani *et al.*, 2009). Osteoporosis is defined as a systemic degenerative disease, which is characterized by decreased bone mass and progressive bone micro-architectural deterioration and results in increasing bone fragility and susceptibility to fractures (Stavros *et al.*, 2013). Emerging evidence has shown that ROS increased bone resorption by enhancing osteoclastic development and activity (Demontrieo *et al.*, 2012; Lee *et al.*, 2005).

Table (5): Comparison of mean  $\pm$ S.E. of ALP, Ca, Mg, Vit. D and PTH serum level between married and unmarried for each low BMD, normal BMD, control groups

			Low BMD	Normal BMD	Control		
ALP U/L	Married	Mean	86.39	80.6	61.88		
		SD	22.68	14.85	19.3		
		SE	$\pm 2.75$	$\pm 3.5$	$\pm 2.87$		
		N	68	18	45		
		Un married	Mean	71.01	82.5	-	
			SD	18.95	27.65	-	
	SE		$\pm 2.708$	$\pm 11.28$	-		
	N		49	6	-		
	Ca Mg/dl		Mean	7.88	9.33	9.26	
			SD	0.607	0.39	0.35	
		SE	$\pm 0.07$	$\pm 0.09$	$\pm 0.05$		
		N	68	18	45		
Unmarried		Mean	7.18	9.26	-		
		SD	0.39	0.26	-		
	SE	$\pm 0.05$	$\pm 0.1$	-			
	N	49	6	-			
	Mg mg/dl	Married	Mean	1.91	1.9	2.00	
			SD	0.11	0.196	0.1	
SE			$\pm 0.01$	$\pm 0.04$	$\pm 0.01$		
N			68	18	45		
Unmarried			Mean	1.94	1.86	-	
			SD	0.13	0.07	-	
		SE	$\pm 0.01$	$\pm 0.03$	-		
		N	49	18	-		
		Vit D ng/ml	Married	Mean	9.9	21.06	28.23
				SD	2.91	7.1	5.64
SE				$\pm 0.35$	$\pm 1.67$	$\pm 0.84$	
N				68	18	45	
Unmarried	Mean			10.12	22.12	-	
	SD			3.24	6.5	-	
	SE		$\pm 0.46$	$\pm 2.67$	-		
	N		49	6	-		
	PTH Pg/ml		Married	Mean	35.34	28.38	40.12
				SD	17.56	8.15	13.11
SE				$\pm 2.13$	$\pm 1.92$	$\pm 1.95$	
N				68	18	45	
Unmarried		Mean		33.19	33.516	-	
		SD		11.23	13.04	-	
		SE	$\pm 1.6$	$\pm 5.32$	-		
		N	49	6	-		

Tables (5 and 6) showed that alkaline phosphatase was higher in osteoporotic married patients than unmarried and maiden patients. Calcium intake from either dietary and/or supplemental sources is a critical nutrient in attaining peak bone mass and for maintaining bone

mass over time (Paik *et al.* 2012). Calcium was significantly lower in unmarried patients than married (Table 5), but the results show a significant higher calcium level in married group than maiden group as shown in Table (6).

Table (6): Comparison of mean  $\pm$ S.E. Ca, Mg, ALP, Vit. D and PTH. Serum level between married and maiden of each low BMD, normal BMD groups

			Low BMD	Menopause	Control		
ALP U/L	married	Mean	86.39	80.6	61.88		
		SD	22.68	14.85	19.3		
		SE	$\pm 2.75$	$\pm 3.5$	$\pm 2.8$		
		N	68	18	45		
	maiden	Mean	65.32	82.5	-		
		SD	15.65	27.65	-		
		SE	$\pm 3.19$	$\pm 11.28$	-		
		N	24	6	-		
		Ca mg/dl	Mean	7.88	9.33	9.26	
			SD	0.607	0.39	0.35	
SE	$\pm 0.07$		$\pm 0.09$	$\pm 0.05$			
N	68		18	45			
maiden	Mean		7.13	9.26	-		
	SD		0.42	0.26	-		
	SE	$\pm 0.086$	$\pm 0.1$	-			
	N	24	6	-			
Mg mg/dl	married	Mean	1.91	1.9	2.0		
		SD	0.11	0.19	0.1		
		SE	$\pm 0.01$	$\pm 0.04$	$\pm 0.01$		
		N	68	18	45		
	maiden	Mean	1.68	1.86	-		
		SD	0.133	0.07	-		
		SE	$\pm 0.027$	$\pm 0.03$	-		
		N	24	6	-		
		Vit. D ng/ml	married	Mean	9.9	21.06	28.23
				SD	2.91	7.1	5.64
SE	$\pm 0.35$			$\pm 1.67$	$\pm 0.84$		
N	68			18	45		
maiden	Mean		10.53	22.12	-		
	SD		3.57	6.5	-		
	SE		$\pm 0.73$	$\pm 2.67$	-		
	N		24	6	-		
	PTH pg/ml		married	Mean	35.34	28.38	40.12
				SD	17.56	8.15	13.11
SE		$\pm 2.13$		$\pm 1.92$	$\pm 1.95$		
N		68		18	45		
maiden		Mean	29.09	33.516	-		
		SD	9.47	13.04	-		
		SE	$\pm 1.93$	$\pm 5.32$	-		
		N	24	6	-		

A tight control of magnesium homeostasis seems to be crucial for bone health. On the basis of experimental and epidemiological studies, both low and high magnesium have harmful effects on the bones (Sara *et al.*, 2013). There was no significant difference between married and unmarried,

osteoporotic patient (Table 5). However in maiden group magnesium level was which is lower than married group as shown in Table (6).

Vitamin D is essential for supporting normal process of absorbing calcium from the intestines. It has been proved that vitamin D and its active

metabolites have a positive effect on the quality of bone mass and cause an increase in their density (Paik *et al.*, 2012). In this study there was no significant difference between married and unmarried patient (Table 5) and maiden (Table 6).

In women, there is some suppression of PTH secretion during the rapid phase of bone loss in early postmenopausal period. In the later stage however there is gradually increasing PTH secretion which increases bone turnover (John *et al.*, 2008; Holick *et al.*, 2006).

There were no significant difference in PTH serum levels between married and unmarried osteoporotic patient (Table 5) While serum level of PTH in married group higher than maiden group.

### Conclusion

Many menopausal women especially unmarried are unaware of their bone density status. The unmarried menopause were experienced unhealthy life style including no healthy food, no exercise no antioxidant uptake and more over facing psychological stress leading to anxiety and depression, so that oxidative stress in various tissues increase due to the release of ROS, leading to the development of a variety of symptoms and pathologies including osteoporosis.

### Acknowledgement

I wish to express my gratitude to Dr. Mohammad Tahir for his help and encouragement with this paper. I would also like to express my thanks to Dr. Taha Ahmed Qaradagy, for his great help in offering all the necessary requirements.

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