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Hematological and Oxidative Stress Markers Analysis for Detection and Prediction of Osteoporosis in Post-menopausal Women

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ABSTRACT

The main purpose of this work was to analyze some biological and oxidative stress markers to predict and diagnose osteoporosis in postmenopausal women. For the experiment, we have chosen 20 healthy menopausal women as control and 20 menopausal women who had osteoporosis. Some biochemical, hematological, and oxidative stress parameters were measured. The sensitivity and specificity of oxidative stress biomarkers in serum, erythrocytes, and leucocytes were estimated using Receiver Operating Characteristics (ROC) curve design. The results of the study demonstrate a significant change (P<0.05) in biochemical and hematological markers during osteoporosis in post-menopausal women. In addition, results show a significant enhancement of MDA content and reduction in GSH, SOD, Catalase, and TAC levels in patients compared to control. Some oxidative stress markers identify as risk factors (P<0.05, OR>1) and represented an important significant specificity (P<0.05) for osteoporosis diagnosis such as serum MDA (AUC=99.6%), erythrocytic GSH (AUC=100%), and leukocytic SOD (AUC=62.5%), with a strong correlation (P<0.05) between GSH levels and serum calcium and ALP changes. In conclusion, several clinical factors contributed to the evolution of osteoporosis in postmenopausal women. Oxidative stress and hematologic markers represent very important diagnostic and predictive factors for osteoporosis in women during the post-menopause period.

Keywords: *Osteoporosis, Post-menopause, Risk factors, Oxidative stress*

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INTRODUCTION

Menopause implies the continual cessation of the monthly cycle and the conclusion of regenerative ability (Weiss *et al*., 2004). The menopausal evolution includes a period of dynamic alterations in non-reproductive and reproductive tissues. This evolution is considered to have a main effect on the etiology of symptoms including night sweats, uterine bleeding problems, vulvovaginal atrophy, hot flashes, and mood changes (Zeidabadi *et al.*, 2020). Osteoporosis is defined as an illness that is specified by disruption of bone microarchitecture, deterioration of bone tissue, and low bone mass: it may result in compromised bone quality and an increment in the chance of breaks (Al-Shali *et al.*, 2019; Nambi *et al.*, 2020; Zerzour *et al.*, 2020). The social and economic burden of osteoporosis is enhancing continuously due to the world population's aging (Sözen *et al.*, 2017). Nowadays, osteoporosis is projected to affect about 14 million adults above 50 years old by the year 2020, affecting above 10 million people in the United States; (Wright *et al.*, 2014). Several late landmarks in scientific studies have indicated that oxidative stress is a significant factor in human beings, resulting in physiological and metabolic modifications and different illnesses in the body (Atoussi *et al.*, 2021). Oxidative stress is an unusual condition as a result of an overabundance generation of oxidants in comparison to the antioxidants (Derouiche *et al.*, 2017) that has been regarded as the major cause of many pathologies (Chetehouna *et al.*, 2020). Minerals have a significant effect on the blood clotting, receiving and sending signals, formation of bones, production of cell energy, oxygen transportation, keeping normal heartbeat, synthesizing and metabolizing proteins and fats, providing immunity to the body, acting as coenzymes, and helping nervous system work properly (Tomlinson *et al.*, 2020). Considering this information, the purpose of our work is based on the determination of the variation and specificity of some oxidative and hematological stress markers in the prediction and diagnosis following up of osteoporosis in menopausal women.

MATERIALS AND METHODS

Subjects and study design

Ethical approval was obtained from the ethics committee (28 EC/DCMB/FNSL/EU2020) of the department of cellular and molecular biology, Faculty of Natural Sciences and Life, El-Oued University. This work was applied to 40 volunteer women aged between 43-54 years, they were divided into two groups; a group of 20 healthy control women with an average

age of 48.26±0.43 years old, a group of 20 menopause women having osteoporosis with the mean age 48.77±0.21 years old. In addition, in this study, we included **v**oluntary women living in the Guemar El-Oued region, who were women suffering from osteoporosis aged from 43 to 54 and like control women were in good health and did not have any pathology. Moreover, in the present research, we excluded all women who were suffering from other acute or chronic pathology and all women using drugs for 30 days or during post-menopausal periods.

Sample collection and analyses

Blood sampling for both groups was done during morning fasting. After the blood sampling, the blood was collected in two types of tubes, in the anticoagulant tube (EDTA), for hematological and oxidative stress (MDA, GSH, SOD, and CAT) parameters assays. In dry tubes, samples were centrifuged at 3000 rpm for 10 minutes, and then the serum was recovered to achieve the dosage of biochemistry parameters: Glucose, calcium, iron, PAL, vitamin C, and total antioxidant ORAC. Serum glucose, calcium, iron, and PAL were determined by the Semi-auto Analyzer Mindray BA-88A and measured using commercial kits from Biomaghreb, (Biomaghreb: glucose-20121, calcium-20051, iron-20064, and PAL-20015. Hematological analysis (FNS) was performed by the hematology Auto analyzer (Mindray).

Oxidative markers measurement

MDA was measured using TBA reagent according to the method described by Sastre *et al.* (2000). The level of reduced Glutathione is determined according to the Weak and Cory (1988). The catalase activity consisting measuring the catalase-induced loss of H_2O_2 contained in the sample according to the method of Aebi (1984). The assay method of SOD activity was the method of Beauchamp and Fridovich, (1971). The plasma vitamin C is measured according to the method of Jagota and Dani (1982) using the Folin reagent. The total antioxidant power of the serum and its capacity to absorb free oxygen radicals (ORAC: Oxygen Radical Absorbance Capacity) were evaluated according to the method of Oyaizu, (1986).

Statistical analysis

Statistical analysis was performed by the SPSSV20.0 software. Results comparisons were carried out by using the Student T-test to contrast means among the groups. Correlation analysis was carried out using the Pearson Correlation test and regression analysis was used for other analyses and statistical data. Differences were considered statically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Description of the study population

The general data of socioeconomic characteristics of the two groups of subjects include age, Body Weight, BMI, social case, number of children, job, educational level, and Blood group. Most of these indicators do not have any statistically significant ($P > 0.05$) differences but serum calcium was decreased in the patients' group compared to the control. Other baseline characteristics between the two main groups are summarized in **Table 1**.

Table 1. Demographic, Clinical and Laboratory Features between the Study Groups

Parameter		Control (n=20)	Patients (n=20)	*P- value*
Age (ys)		48.26±0.43	48.77±0.21	0.114
Body Weight (kg)		68.60±0.243	71.40±0.61	0.080
Height (cm)		162.76±0.164	161.98±0.130	0.204
Body Mass index		26.01±0.182	27.39±0.108	0.152
Blood glucose (g/l)		0.82±0.023	0.92±0.018	0.009
Serum Calcium (g/l)		74.31±1.08	65.15±2.04	0.006
Serum ALP (U/l)		129.0±10.2	182±13.9	0.002
Number of children		5,333±0,18	6,06±0,18	0,081
Social Case (%)	Married	81	96	0.014
	Single	7	2	0.024
	Divorced	10	1	0.000
	Widow	2	1	0.072
Job (%)	Worker	25	10	0.000
	Housewife	75	90	0.000
Educational Level (%)	Illiterate	13	14	0.062
	Primary	15	28	0.002
	Junior high	26	24	0.123
	High School	26	23	0.092
	Higher education	20	11	0.014
Blood Group (%)	A	43.33	20	0.000
	B	10	10	0.231
	AB	6.67	13	0.002
	O	40	57	0.051

Hematological parameters

The illustrated results of the hematological parameters in **(Table 2)** show that in the osteoporosis patients group there was a significant increase (P<0.05) in WBC, Lymphocytes, RBC, and in HCT and PLT (P0.001>) levels and no significant change of hemoglobin level in comparison with the control group.

Table 2. Changes in the Haematological Levels in Control and Osteoporosis Patients

Parameter	Control (n=20)	patients (n=20)	P-*value*
White Blood Cells (×10³/µl)	5,669±0,286	7,169±0,56	0,020
Lymphocytes (×10³/µ)	2,042±0,073	2,200±0,195	0,432
Hemoglobin (g/dl)	125,73±2,49	130,06±1,83	0,032
Red Blood Cells (×10⁶ /µl)	4,562±0,055	4,752±0,086	0,043
Hematocrite (%)	39,8±0,719	43,256±0,603	0,000
Platelets (×10³/µl)	136,06±1,86	255,6±16,4	0,000

Oxidative stress markers

The analysis of blood oxidative stress parameters in control and patient are shown in **Table 3**, The results show a significant decrease of GSH level in erythrocyte (P<0.001), leukocyte (P<0.001) and serum (P<0.001) and also decrease of catalase and SOD activities in leukocyte (P<0.001) and in serum (P<0.001, P<0.05) and TAC activity in serum and a significant increase of MDA level in leukocyte (P<0.05) and serum (P<0.001) in osteoporosis patients group in comparison to the control group.

Table 3. Parameters of Oxidative Stress in Blood of Control and Osteoporosis Patients

Parameter		Control (n=20)	Patient (n=20)	*P-value*
MDA (nM/mg of Hb)	Erythrocytes	9,06±1,03	7,587±0,780	0,083
	Leukocytes	7,566±0,785	12,11±1,76	0,021
	Serum	28,02±2,84	92,5±10,4	0,000
GSH (nM/mg of Hb)	Erythrocytes	1,065±0,041	0,898± 0,027	0,000
	Leukocytes	1,448±0,217	0,765±0,107	0,000
	Serum	3,751±0,146	2,969±0,295	0,021
CAT (UI/g of Hb)	Leukocytes	0,032±0,003	0,021±0,001	0,000
	Serum	0,11±0,006	0,099±0,004	0,031
SOD (U/mg of Hb)	Leukocytes	15,250±0,423	12,929±0,339	0,000
	Serum	78,56±1,99	73,43±1,03	0,000
Vit C (µg/ml)	Serum	61,63±3,47	66,38±3,86	0,238
TAC (U/l)	Serum	21,24±1,55	14,88±1,69	0,002

MDA=Malondialdehyde; GSH=Glutathione; CAT= Catalase SOD=Superoxide dismutase; TAC=Total antioxidant capacity; Vit C =Vitamin C.

Study of odds ratio values of biochemical and oxidative stress markers

Odds Ratio (OR) values for some oxidative stress parameters and biochemical markers of controls and patients groups **(Table 4)** show that decreased serum ORAC, leukocyte SOD, erythrocyte GSH, leukocyte GSH, leukocyte catalase, and serum calcium are shown to be important risk factors for osteoporosis OR (6.303-106.375) with P<0.05. Reciprocally, decreased serum MDA and serum PAL are protective factors against osteoporosis in the study population (OR=0.032; P=0.000, OR=0.084; P=0.000), respectively.

Table 4. Odds Ratio Value of Biochemical and Oxidative Stress Markers

	Control %	Patient %	OR	CI95%	*P-value*
Serum ORAC			6.303	2.604-15.255	0.000
Positive	32	11			
Negative	18	39			
Leukocyte SOD			106.375	13.475-839.745	0.000
Positive	37	01			
Negative	16	46			
Erythrocyte GSH			37.161	4.796-287.936	0.000
Positive	32	01			
Negative	31	36			
Leukocyte GSH			18.951	2.428-147.909	0.000
Positive	21	01			

Negative	41	37			
Leukocyte catalase			8.957	2.424-33.103	0.000
Positive	17	3			
Negative	31	49			
Serum MDA			0.032	0.004-0.248	0.000
Positive	30	50			
Negative	19	1			
Serum calcium			18.222	5.035-65.946	0.000
Positive	32	3			
Negative	24	41			
Serum PAL			0.084	0.030-0.233	0.000
Positive	19	44			
Negative	31	6			

Predictive factors study

The results obtained **(Figure 1 and Table 5)** show that serum MDA, Erythrocytic GSH, and Leukocytic SOD levels are the highest percentage of specificity (100%) and important percentage of sensitivity (50.0, 43.8, 75%) respectively for women with osteoporosis. In addition, Serum TAC, Leukocyte GSH, and Leukocytic catalase were significant predictive factors for osteoporosis in postmenopausal women.

Table 5. Sensitivity, Specificity and AUC Values of Biological Markers for Women with Osteoporosis

Test Result Variable(s)	Sensitivity (%)	Specificity (%)	AUC (%)	SE	CI_{95}%		*P-value*
Serum TAC	50.0	25	19.1	0.076	0.043	0.340	**0.003**
Leukocytic SOD	75.0	100	62.5	0.063	0.001	0.249	**0.000**
Serum SOD	68.8	31.2	32.2	0.099	0.129	0.516	0.086
Erythrocytic GSH	43.8	100	100	0.000	0.000	0.000	**0.000**
Leukocyte GSH	18.8	25	10.5	0.058	0.000	0.219	**0.000**
Serum GSH	81.3	12.5	42.8	0.103	0.226	0.630	0.486
Serum vitamin C	43.8	62.5	58.6	0.103	0.384	0.788	0.407
Leukocytic catalase	50.0	62	15.8	0.071	0.019	0.298	**0.001**
Serum catalase	31.3	43.7	30.9	0.096	0.121	0.496	0.065
Erythrocytic MDA	43.8	25	34.0	0.100	0.145	0.535	0.122
Leukocytic MDA	43.8	100	70.7	0.098	0.515	0.899	0.064
Serum MDA	50.0	100	99.6	0.006	0.983	1.000	**0.000**

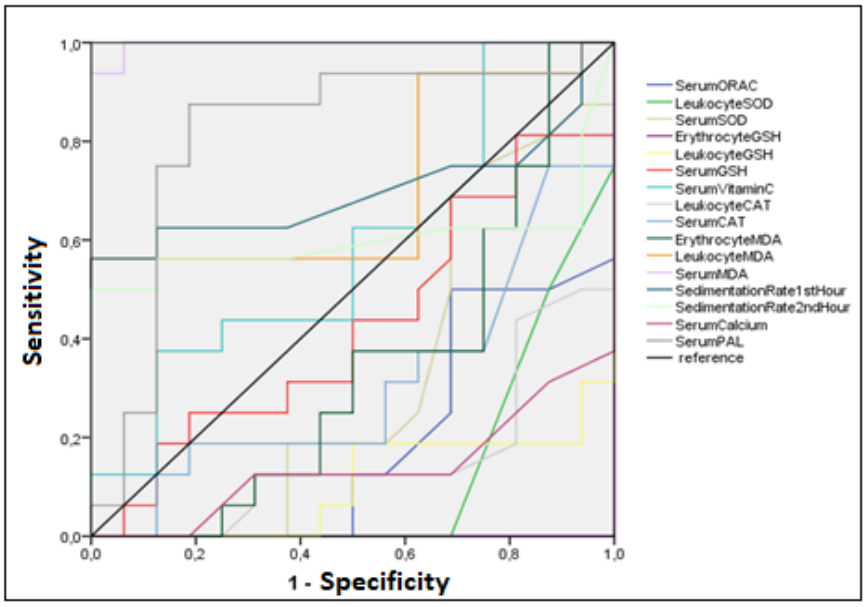

Figure 1. ROC Curve for Biological Markers in Women with Osteoporosis

Correlation between biological markers

The results **(Table 6)** represent the correlation between oxidative stress parameters (serum ORAC, leukocyte SOD, erythrocyte GSH, leukocyte GSH, leukocyte catalase, and serum MDA), hematological parameters (WBC, HGB, RBC, HCT, PLT), and biochemical parameters (calcium, ALP) in patients group with osteoporosis. There was a positive correlation (P<0.05) between erythrocyte GSH and calcium (P=0,005 and R=0,381), erythrocyte GSH and ALP (P=0,047 and R=0,393), Leukocyte GSH and serum calcium (P=0,046 and R=0,34). There was no correlation (P>0.05) between the rest of the correlation test in the patients' groups.

Table 6. Correlation between Biological Markers for Women with Osteoporosis

Parameters		WBC	HGB	RBC	HCT	PLT	Serum Calcium	Serum ALP
Serum ORAC	P	0,926	0.565	0,729	0,642	0,429	0,447	0,734
	R	0,019	0.118	0,071	0,096	0,162	-0,108	0,070
Leukocyte SOD	P	0,580	0,879	0,582	0,420	0,644	0,435	0,146
	R	-0,114	-0,031	-0,113	-0,165	-0,095	0,111	-0,293
Erythrocyte GSH	P	0,624	0,221	0,665	0,303	0,617	**0,005**	**0,047**
	R	-0,101	-0,248	-0,089	-0,210	0,103	0,381	0,393
Leukocyte GSH	P	0,458	0,463	0,929	0,700	0,320	**0,046**	0,955
	R	0,152	-0,151	-0,018	-0,079	0,203	0,340	-0,012
Leukocyte Catalase	P	0,318	0,379	0,962	0,722	0,761	0,375	0,115
	R	-0,204	-0,180	-0,010	-0,073	-0,063	-0,126	0,317
Serum MDA	P	0,938	0,870	0,715	0,619	0,285	0,582	0,521
	R	0,016	0,034	-0,075	0,102	-0,218	0,078	-0,132

The obtained results show a significant increase in PAL level in the patient group as compared to control. This result is in line with the research of Pardhe *et al.*, (2017), who show that the PAL level was slightly higher in the post-menopausal group. All Bone Mineral Density (BMD) results were remarkably reduced by PAL increment, while bone-specific alkaline phosphatase, is an indicator of bone turnover and formation and is utilized in the skeletal status evaluation (Hailing *et al.*, 2018). On the other hand, we found a significant decrease in calcium level in the patients' group as compared to the control group. This result is consistent with the research of (Beto, 2015), whose findings demonstrated that the serum calcium level was significantly lower in the post-menopausal group. Calcium plays a key role in human physiology. As a main constituent of the mineral component calcium provides stiffness to the collagen network of the mature bone. Inadequate calcium accrual, resulting in a sub-optimal bone mass peak and low bone mineralization, is a significant parameter favoring osteoporosis and fracture (Kelly *et al.*, 2020). The results of hematological parameters indicated a remarkable increment in WBC, HGB, HCT, and PLT in the osteoporosis patients group in comparison to the control group. These results support a possible linkage between bone metabolism and hematopoiesis. Hematopoiesis is the process by which immature blood cells develop into mature cells (Paspaliaris & Kolios, 2019). According to Schyrr *et al.,* (2018), differences in the osteoporotic bone microenvironment translate into altered dynamics upon hematopoietic stress. Moreover, Valderrábano *et al.,* (2018) found that low bone health would result in enhanced cells of myeloid lineage such as neutrophils and monocytes. Osteoblastic lineage cells might affect neutrophils and monocytes differentiation. The enhancement in neutrophils can be associated with the chronic inflammation that happens with aging. The results of the oxidative stress study showed for the osteoporosis patients group a very high significant increase in serum MDA level as compared to the control women. The results showed that serum MDA has a high specificity in ROC statistics, which showed the importance of MDA in the prognostic of osteoporosis. The results found were similar to those observed in the study of Berköz *et al.* (2017) which showed that serum MDA levels were significantly higher in postmenopausal women with osteoporosis than in the healthy controls. Sakuraba *et al.*, (2020) reported that MDA had an osteoclastic activity. Our results show that leukocyte MDA is significantly increased in the osteoporosis patients group as compared to control women, with high specificity in the ROC statistic test, which showed the importance of this parameter in the identification of the disease. These results are supported by Ahmedian *et al.,* (2017) study. Raghavan *et al.* (2012) found that MDA could significantly induce key inflammatory cytokine expression in lymphocyte via oxidant stress, signaling pathways (p38MAPK), and transcriptional factors (NF-κB), which in turn enhance lymphocyte activation. The results also show that for the osteoporosis patients group, a very high significant decrease in leukocyte and serum SOD level (P<0.001) in comparison to that in the control. Depressing actions of the antioxidant enzymes such as SOD indicated a defense mechanism that has been overwhelmed in mitigating the enhanced superoxide production by the osteoclasts showed by enhanced contents of MDA in the serum (Kuyumcu & Aycan, 2018) and it might cause markedly increased bone demineralization and, as a result, may increase destructive free radical levels (Bacou *et al.,* 2021). The results show that for the osteoporosis patients group, a highly significant decrease in serum TAC level (P<0.01) as compared to that in the control. TAC method is relevant to in vivo conditions since it uses a biologically relevant free radical source (peroxyl radical) that is the highest common free radical in human biology. It considers both degrees of inhibition and inhibition time of free radical action as a result

of antioxidants (Hunyadi, 2019). The results of the oxidative stress study showed that there is a significant decrease in GSH, catalase, and SOD level of WBC, in GSH level of RBC, and both of GSH and catalase level of serum in the patients' group compared to controls. Glutathione (GSH) is a non-enzymatic antioxidant that aids the defense mechanism against oxidative stress created by free radicals (Derouiche *et al.*, 2019). To reduce the cell-damaging impacts of ROS (reactive oxygen species), aerobic organisms evolved by expressing different antioxidant defenses, such as catalase. The mechanisms by which cells sense H2O2 and $O^{2\bullet}$ are not comprehended; however, many transcriptional parameters that adjust the expression of antioxidant genes are adjusted by decrease counteractions and oxidation (Tonelli *et al.*, 2018). Intracellular Redox Imbalance resulting from SOD shortage has a vital impact on the progress and development ion of bone fragility both in vitro and in vivo (Tan *et al.,* 2018).

CONCLUSION

This study indicates that hematological parameters change and Oxidative stress were correlated with the osteoporosis disease of menopause in women as a reason or as a developmental factor for it. And through the ROC analysis results MDA, GSH, SOD, and TAC were considered to be the most important markers which contribute in the early detection of osteoporosis disease in post-menopause women.

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CONFLICT OF INTEREST: None

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