

## دراسة مقارنة لمستويات الشد التأكسدي وأكسدة الدهون وفيتامين E في أمصال كبار السن المصابين بالفصام العظمي والأصحاء

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### الخلاصة

ان أنتاج أصناف الأوكسجين الفعالة في معظم الأمراض ومن ضمنها الفصام العظمي (OA) تليلاً على أنه من أكثر العمليات الضارة بخلايا الجسم .ولأجل التحري عن دور الجذور الحرة عند مرضى الفصام العظمي وذلك بمقارنة المألون ثنائي الألديهيد (MDA) تليلاً على أكسدة الدهون ومستوى فيتامين E الذي يعد من مضادات الأكسدة عالية الكفاءة في امصال المرضى مع مجموعة من الأصحاء .

شملت الدراسة 34 مريضاً بالفصام العظمي للركبة الذين تم تشخيصهم باستخدام تقنيات تشخيصية مختلفة ( 18 ذكراً و 16 إناثاً ) تتراوح أعمارهم بين 45-78 سنة كذلك شملت الدراسة 32 من الأصحاء (16 ذكراً و 16 إناثاً). أظهرت نتائج الدراسة إن هناك زيادة معنوية في مستوى الـ MDA في مصل مجموعتي المرضى (النساء والرجال) مقارنة مع مجموعة السيطرة المقاربة لأعمار المرضى. كذلك وجد انخفاضاً معنوياً في مستوى فيتامين E لمجموعتي المرضى مقارنة مع مجاميع الأصحاء .

# **Comparative Study of Oxidative Stress, Lipid Peroxidation Marker, and Vit E Levels in Sera of Elderly Osteoarthritis Patients and Healthy Control.**

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## **Abstract**

The production of reactive oxygen species in most diseases including osteoarthritis (OA) is confirmed to be the most destructive process to human cells.

To investigate the role of free radicals in patients with OA and by comparing lipid peroxidation marker malondialdehyde (MDA) and Vit E as one of the potent antioxidant in Sera of (OA) patients with those in healthy population.

The study includes 34 patients with knee OA diagnosed by doctors using different diagnostic parameters techniques, 18 females and 16 males and their age ranged 45 – 78 years, in addition to 32 healthy control 16 males and 16 females. The result revealed a significant elevation in serum MDA sera in both patient groups (male and female) compared to match aged healthy control groups. On the other hand a significant decrease in serum Vit E levels in both patient groups compared to control groups, was noticed.

## **Introduction**

The energetic benefit of aerobic metabolism is associated with generation of free radicals (FRS). These radicals are highly reactive molecules generated by the redox reaction that occur as part of normal cell metabolism, further FRS may be formed by some reactive enzymes[1].

Lipid peroxidation is a well established Mechanism of cellular injury in human, and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, which are the most abundant malondialdehyde (MAD) which is a highly reactive bifunctional molecule, and has been shown to crosslink erythrocyte phospholipids and proteins. This process results in impairment of the membrane-related functions that ultimately leads to diminished survival. Therefore, measurement of malondialdehyde is widely used as an end product and indicator of lipid peroxidation. Increased levels of lipid peroxidation products have been associated with a variety of disease in humans (Voet and Voet, 2004) [2].

On the other hand FRS could be generated by exposure to environmental factors, also produce by activated phagocytes, and neutrophils to kill pathogens[3]. The generation of reactive oxygen species (ROS) via (FRS) formation are capable of damaging the biologically relevant molecules such as lipids, proteins, carbohydrate, and DNA[4]. These processes may be involved in the initiation and propagation steps of several age related diseases[5].

Osteoarthritis (OA) is a degenerative joint disease. It results from the combination of genetic abnormalities and joint disease[6].

Among the multiple risk factors have been linked to OA in epidemiology studies age and

female versus male[7].

Aerobic cell have the capacity to remove the active product of oxygen through endogenous cellular enzymatic defenses, additional cellular defenses include small antioxidant molecules i.e.  $\alpha$ -Tocopherol (vitamin E) is the most widely distributed antioxidant in nature, when vitamin E donated an electron to a lipid peroxy radical it is converted to free radical stabilized by resonance structure.[8]

The major function of Vit E in the body is an antioxidant and it acts primarily by scavenging active oxygen free radicals. It also protects other antioxidant from being oxidized. The capability is then also great in helping to prevent the degenerative diseases[9].

The role of diet (composition of rich antioxidant sources food) in the treatment of OA remains unclear. Current research into diet as an independent factor is focused on the merits of antioxidants (i.e. Vitamin)(Kalunian and Brion[10],( 2005)

Since OA is by far the most common degenerative joint disorder throughout the world, and is one of the leading causes of disability and pain in elderly, and the prevalence of this disease is less than 0.1% in those aged 25 years old versus a rate of over 60% in people over age 55 years[11].

So this study conducted on elderly people more than middle aged.

## Subjects and Methods

The study was performed on (34) randomly selected patients (18 females and 16 males) with OA of the Knee, at the outpatient clinic of Baghdad Teaching hospital in Baghdad-Iraq.

The selected patients have symptomatic and radio logic evidence of OA in one or both knee joints, and show different signs and symptoms. The control group includes 32 healthy persons (16 females and 16 males).

- 1 Blood samples have been withdrawn from the study subjects by uenipuncture at 10am., in a disposable tubes and centrifuged (250 xg for 10 min) within 45 min after collection to get the serum which was stored at  $-20^{\circ}\text{C}$ , unless used directly.
- 2 Determination of serum MDA:- MDA was measured according to[12]by monitoring the colored complex formed by the reaction of MDA with thiobarbituric acid the absorbance was taken at 532 nm.
- 3 Determination of serum  $\alpha$ -Tocopherol (vitamin E): High performance liquid chromatography (HPLc) technique, provided with UV detector was used[13].

The serum was deproteinized with ethanol; the vitamin and its ester were extracted by hexane, dried, and redissolved in chloroform prior to injection on the silica column. The vitamin was eluted with 99% hexane and monitored at 287 nm.

Antioxidant solution was used for the presentation of  $\alpha$ -Tocopheroloxidation during extraction; all steps were performed under stream of nitrogen.

Student -t-test was used to compare the significance of difference in the mean values in comparison groups ,  $p < 0.05$  was considered statically significant.

## Result and Discussion

Table (1) showed the results of lipid peroxidation marker (MDA) in Sera of control groups and patient with Osteo arthritis respectively.

No significant differences were found in serum MDA levels between male and female groups in healthy controls while a significant increase in serum MDA of male patients compared to female patients was noticed. From table (3)which showed significant differences between serum MDA levels of male patients ( $117.69 \pm 11.12$ ) compared to serum level of control male

groups ( $35.3 \pm 4.3$ ) also a significant increase in females patients ( $112.5 \pm 0.9$ ) compared patients group to that of healthy female ( $33.9 \pm 5.9$ ).

Oxidative damages are implicated in most diseases, the level of MDA which is one of some product of lipid peroxidation used as marker of free radicals oxidation of lipid. The effect of these radicals on joints of humans has been confirmed [14,15].

Tables (2) and (4) showed serum level of vit E in control groups, patients with OA groups and serum level of vit E in studied groups respectively. No significant difference between vit. E levels in female and male in control groups also a non significant decrease in female patients compared to male patients was found. A significant reduction in vit E levels in female patients ( $0.32 \pm 0.03$ ) compared to control ( $0.85 \pm 0.08$ ) and a significant reduction in Vit E of male patients ( $0.38 \pm 0.03$ ) compared to male control group ( $0.85 \pm 0.08$ ) was noticed.

The reduction in Vit E in female and male groups compared to the healthy controls and the decline of this Vit may promote the degenerative process in the patient groups [16,17].

The increase in free radicals generation is due to the disease stage or the impairment of some antioxidant defenses and this agreed with other results reported that raise in (MDA) could be due to increase generation the active oxygen species due to the exclusive oxidative damage generated in these patients. The oxygen species in turn can oxidize many other important biomolecular including membranes lipid [18].

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**Table (1) MDA&Vitamin E levels of all studied groups**

Subjects	No.	MDA mg/dL	P value	Vit E mg/dL	P value
Te	32	34±5.1		0.850±0.075	
Fe	16	33.9±5.9	Ns<0.05	0.850±0.075	Ns<0.05
Me	16	35.3±4.3	Ns<0.05	0.850±0.075	Ns<0.05
TOA	34	115.095 ± 11.0		0.35± 0.03	
FOA	18	112.5± 10.9	S<0.05	0.32± 0.028	Ns< 0.05
MOA	16	117.69± 11.12	S<0.05	0.38± 0.031	Ns< 0.05

Note: All Values shown in tables are mean ± SEM

Te = Total healthy control.

Fe = Female healthy control.

Me = Male healthy control.

TOA = Total patient with Knee Osteoarthritis.

FOA = Female with knee Osteoarthritis.

MOA = Male with Knee Osteoarthritis.

S = Significant p. value.

NS = Not significant p. value.

**Table (2) MDA & Vitamin E levels in sera of controls and patients groups.**

Subjects	MDA mg/dL	Vit E mg/dl	P values
Fe	33.9±5.9	0.85± 0.08	S<0.05
Me	35.3±4.3	0.85± 0.08	S<0.05
FOA	112± 0.9	0.32± 0.03	S<0.05
MOA	117.69± 11.12	0.38± 0.03	S<0.05