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Determination of paraoxonase enzyme and zinc in women suffering from arthritis in the city of Balad

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Abstract:
The study was conducted at Ballard General Hospital and Ballard Private Laboratory for Pathological Analysis on female patients with arthritis, and 90 blood samples were collected after careful diagnosis by specialists based on clinical symptoms and pathological examination sample, including the control group. (30) sampled women with arthritis during the study period from November 2023 to April 2024 (60). The age group for affected women is 35-60 years, and the age group for healthy women is 35-60 years. year. The findings demonstrated that female arthritis patients had significantly lower blood zinc levels ($P<0.05$) in their serum than the healthy group, and that the levels of the enzyme paraoxygenase in their blood serum were significantly lower at the probability level compared to the control group ($P<0.01$).

Keywords: Arthritis, Paraoxonase , Zinc**.**

1. Introduction

Arthritis is a common disease in contemporary and modern medicine, and the presence and distribution of the disease in ancient bones suggests that it occurred 3000 years ago in North America [1]. The disease was first described in France in 1800 AD when nine women were diagnosed with the disease, which is believed to be a form of gout and is known as primitive gouty asthenia. **Paraoxonase** (EC.3.1.8.1) belong to the hydrolase and esterase families, classified according to their reaction with organophosphorus compounds [2]. The Paraoxonase family consists of three genes: PON1, PIN2, and PON1. PON3 and the enzyme (PON1)[3]. are the most researched and have not been considered. The fact that PON2, the eldest of them, descended from PON3, a multigene family of lipolactonases, is inevitably more significant. This gene can be found on chromosome 7 of humans. and its protein structure is similar in that it hydrolyzes diethyl p-nitrophenyl phosphate, a polarized form of the organophosphorous pesticide. Parathion [4]. **Zinc** is an essential element for terrestrial life. Due to its requirement as a structural component or interaction site for many proteins, the zinc-binding fraction is highly conserved among species [5]. Zinc sites in proteins consist of polyhedral Zn with S, N, or O heads attached to cysteine, histidine, glutamate, aspartate, and water. The coordination number of zinc ranges from 4 to 4 for structural zinc binding. In the case of r reactive sites containing O and N as single atoms, the number of thiol groups derived from cysteines reaches 6 [6]. There has been an increase in the number of patients presenting to outpatient clinics with an abnormal taste or smell, often accompanied by hypoglycemia or abnormality. It is estimated that approximately 140,000 new patients with these symptoms have been registered. Each year, approximately 30% of these patients suffer from dietary zinc deficiency, The regular zinc consumption may not be adequate to fulfill the daily demands of some groups (children, the elderly, young women on weight-loss regimens, and others. These individuals may suffer from near or genuine zinc insufficiency [7].

2. Materials and Methods:

2.1. Samples of study.

The study included samples from (90) women with arthritis and (30) healthy individuals (control group) at Al-Balad General Hospital and a group of public laboratories in the Al-Balad region between November 2023 and April 2024 (sample). The ages of the infected people range between 35-60 years and the healthy people between 35-60 years. The patients were diagnosed by experts from Al-Balad General Hospital and private laboratories, and special forms were organized to collect information. About infected patients.

2.2. Collection and preparation Of Blood Samples

Using a disposable syringe to extract blood from an arm or elbow vein, samples were taken from each subject. The volume of blood extracted ranges from 2 to 5 milliliters, depending on the health status of the patient.

2.3. Determination of Paraoxonase1 in blood serum[8].

The enzyme paraoxonase 1 (PON1) was measured using a ready-made analysis kit from the Chinese company Ssangyong, and it relied on the immune-linked enzyme assay technology and based on the sandwich principle, using two antibodies that bind to the enzyme protein.

A. **Procedure**

I left all reagents at room temperature for half an hour before use and prepared the next solution

Then I took the next action steps.

. 1 Add (50 ml) of the standard solution to the well appointed standard solution on the plate and no antibody is added as it was previously added to the standard solution and 50 ml of the HRP-binding reagent solution.

Table 1 shows the concentrations of standard solutions

Conc.	Number	Contenets
30 IU/L	Standard No. 1	300?l Standard No.1+ 150µl Standard diluents
20 IU/L	Standard No. 2	300 µl Standard No.1 + 150µl Standard diluent
10 IU/L	Standard No. 3	150 µl Standard No.2 + 150µl Standard diluent
5 IU/L	Standard No. 4	150 µl Standard No.3 + 150µl Standard diluent
2.5 IU/L	Standard No. 5	150 µl Standard No.3 + 150µl Standard diluent

2. Add (40 ml) serum to the designated sample well, then add (10 ml) enzymatic antibody and (50 ml) HRP-conjugate solution.

3. Do not add enzymatic antibodies and HRP-conjugation reagent solution to the blank.

4. Cover the dish and incubate at 370°C for 60 minutes.

5. Remove the cover of the dish, empty its contents, then inject 0.35 ml of washing solution, leave it for 30 seconds, then empty it and repeat the washing process 5 times.

Then empty the contents of the dish and pat it vigorously with paper towels after the final wash

6. Add 50 mL of substrate A to each well, then add the substrate solution B, cover and incubate at 370 °C in the dark for 10 minutes.

7. Add the reaction stop solution (50 ml) to all the wells and note that the color in the wells changes immediately from blue to yellow after addition.

8. Immediately use an instrument to measure the absorbance of all etches at the wavelength (450 nm) within 10 minutes after adding the reaction stop solution.

B. Calculations

Draw a standard curve between the absorbance value of the standard solution and its concentration on the Y axis.

2.4. Estimation of zinc concentration in blood serum[9].

When zinc and chromophores in the reagent combine, colorful compounds are created, and the strength of the color corresponds to the amount of zinc in the sample.

A. Reagents

Reagent A: Borate buffer 0.37 M, pH 8.2, salicyl oxime 1.25 mM, surfactant and preservative.

Detector setup:

Add 2 ml of reagent B to a bottle of reagent A stored at (2-8)°C.

B. Procedure

Mix at room temperature and read absorbance at 560 nm after 5 minutes. Color stays put for 30 minutes.

Reagents	Blank	Standard	Sample
Work Reagent	1 ml	1 ml	1 ml
Distilled Water	50 µl	–	–
Standard	–	50 µl	–
Sample	–	–	50 µl

C. **Calculations**

The zinc concentration is calculated as follows:

$$\text{Zn µl/dl}=[A_{sample} / A_{standard}\ ] \times 200$$

$$\text{Zn µmol/dl}=[A_{sample} / A_{standard}\ ] \times 30.6$$

3. Results and discussions

3.1. The level of paraoxonase enzyme in the blood of patients and healthy people

The table shows that the mean ± standard deviation of paraoxonase in the control group was (1.81 ± 1.40), while the mean ± standard deviation of paraoxonase in the arthritis patient group was (1.73 ± 1.08).

Table (2) Mean ± standard deviation of paraoxinase in the sera of the studied samples

Group parameter	Control	Patient	P≤
	Mean± S.D		
NO.	30	60	
PON-1 (mg/ml)	1.81+1.40	1.73+1.08	**0.01**

The blood paraoxygenase levels in female arthritis patients significantly decreased at the probability level (P<0.01) in comparison to the control group, as indicated by the above results, as depicted in the figure below:

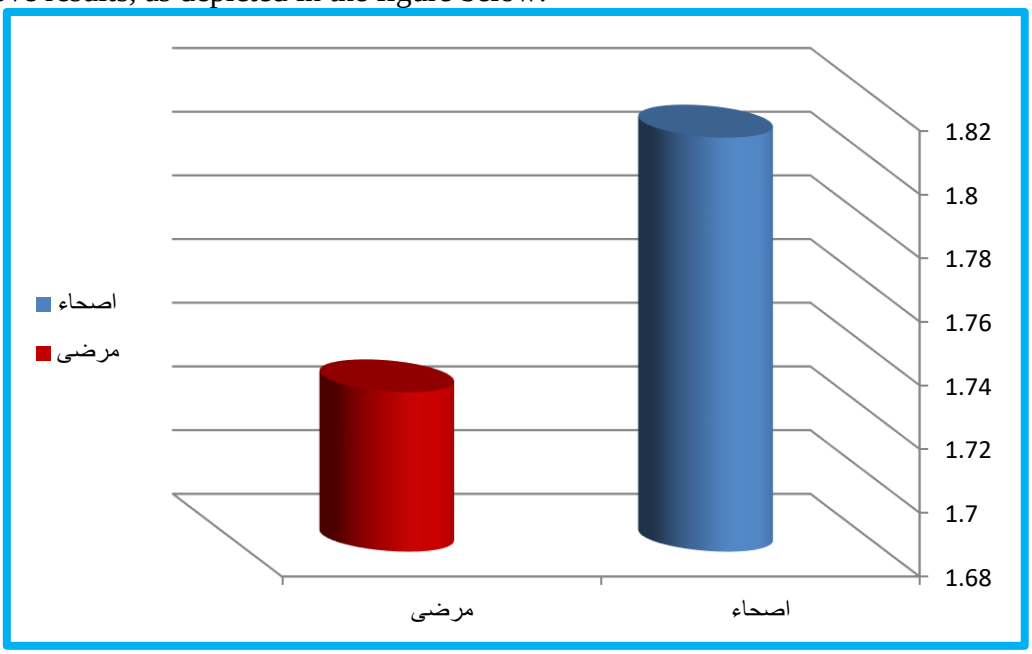

Figure (1) represents the average standard deviation for the paraoxonase enzyme

That study's findings are supported by the present investigation (Abd, H. N., & Hasan, A. B. 2021). According to a study, PON-1 activity is linked to arthritis because PON-1 levels were much lower in RA patients than in controls. cardiovascular disease and arthritis are related[10].

The current study is also consistent with the study of (Al-Banna et al. 2014). The current study concluded that "patients with rheumatoid arthritis have lower levels of the enzyme PONI, which is associated with decreased antioxidant potential of PON-1 activity" and thus decreased PON-1 activity may play a role in cardiovascular disease caused by arthritis[11]. Research by Charles Schuman (2013) and Rodriguez Carrillo (2016) revealed that while genetic variants are known to control PON-1 activity, there is no correlation between RA and cardiovascular disease. Other elements might change

how it functions. It has been discovered that the development of dyslipidemia and cardiovascular illness in patients with rheumatoid arthritis is linked to anti-HDL antibodies. The purpose of this study was to investigate the roles that PON-1 activity, anti-HDL antibodies, and PON1 genetic variations play in cardiovascular disease in rheumatoid arthritis patients [12-14]. Beans (Wu, 2018). Reduced PON-1 activity in patients with rheumatoid arthritis may accelerate the development of atherosclerosis in patients with arthritis [15]. Studies by (Al-Banna et al., 2014 and Tang (2012)) showed that in patients with arthritis, PONI activity was lower than in healthy individuals, with a consequent increase in peroxide production. Lipids or other indicators of oxidative stress [16,17]. (Shahem Mohammad Nejad et al., 2014). Reactive oxygen species are produced in excess by activated inflammatory cells in RA patients, which causes oxidative stress and tissue damage [18]. Reduced PON-1 enzyme activity has been linked to tissue and microvascular complications in arthritis [19]. It's also hypothesized that preserving serum PON-1 activity in RA patients with therapeutic antioxidant concentrations might stop vascular damage. According to a research by Pascol, Golden, et al., PON and arthritis had the opposite relationship. These findings could suggest that reduced PONI activity in rheumatoid arthritis is caused by changes in oxidant/antioxidant status [20]. and earlier research has hypothesized that elevated levels of PONI protein damage might be the cause of patients' reduced PONI activity in rheumatoid arthritis patients. Reactive oxygen species levels are generated by RA patients as opposed to being inadequately synthesised [21].

3.2. Measuring the level of zinc in blood serum.

The table shows that the mean ± standard deviation of zinc in the control group was (131.9 ± 13.6), while the mean ± standard deviation in the arthritis patients group was (162.9 ± 19.4).

Table (3): Mean ± standard deviation of zinc in the sera of the studied samples

Group parameter	Control	Patient	P≤
	Mean± S.D		
NO.	30	60	….
Zinc(mg/ml)	131.9+13.6	162.9+19.4	0.05

The results showed that compared to the healthy group, the level of zinc in the blood of the group of arthritis patients was significantly lower at the probability level (P<0.05), as shown in the figure below:

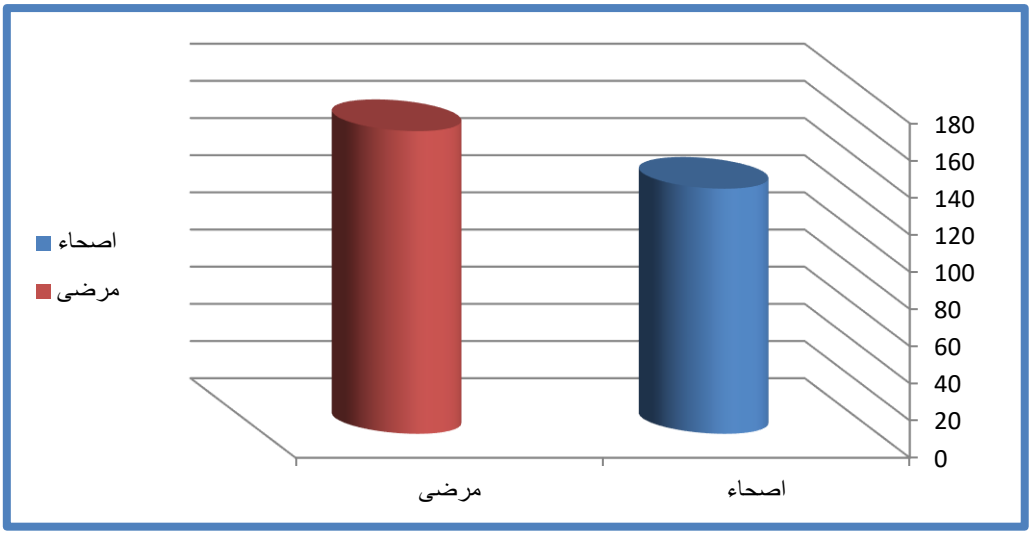

Figure (2) represents the average standard deviation for Zinc

While arthritic patients had lower zinc amounts than controls, these findings are in line with other studies (Yang, 2023). Since the majority of zinc in the body is kept in the bones, zinc is essential for many physiological functions, including bone homeostasis. Zinc is a crucial cofactor for several proteins involved in microstructural stability and bone remodeling in addition to being a component of bone [22].

According to Shaw's (2023) research, individuals with rheumatoid arthritis may have reduced blood zinc levels because pro-inflammatory cytokines have enhanced the entrance of zinc into cells. Thus, through epigenetic pathways, zinc deficiency increases the release of pro-inflammatory mediators and reactive oxygen species (ROS), which in turn promotes inflammation [23].

(Nigi and others, 2012). Zinc deficiency is therefore linked to improper B and T cell maturation and function [24], an unbalanced ratio between type 2 Th2 and type 1 Thl T cells, and compromised natural killer cell activity, which is related to immune system recognition cells. body being infected by viruses or bacteria [25].

4. Conclusions

Compared with healthy subjects, the serum levels of paraoxygenase in women with arthritis are significantly reduced. Zinc levels in women with arthritis were significantly lower compared to the healthy group.

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