

Determination Of Superoxide Dismutase (SOD) ,Catalase and Alkaline Phosphatase (ALP) Levels in Rheumatoid Arthritis Patients

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Abstract

I have been studied the direct effects of superoxide dismutase (SOD) and catalase activity on function in human bones in rheumatoid arthritis patients. This study including (fifteen) rheumatoid arthritis patients and (fifteen) healthy control that aged 25-35 years in both them. The results show significantly increase in superoxide dismutase (SOD) and significantly decreased in catalase activity in patients compared with healthy control P-value(0.00,0.001).So the results obtained significantly increased in alkaline phosphatase (ALP) levels in patients compared with healthy control (p=0.00).Sex difference no effect on SOD,CAT and ALP levels in rheumatoid arthritis patients.

الخلاصة

في هذه الدراسة تم قياس مستويات كلا من السوبر اوكسيد دسميوتيز والكاتليزوبيان علاقتها بالجذور الحرة في مصل المصابين بالتهاب المفاصل الروماتزمي،تضمنت الدراسة ١٥ شخص مصاب تتراوح أعمارهم بين (٢٥ إلى ٣٥) سنة مقارنة مع ١٥ شخص اصحاء كمجموعة سيطرة تتراوح أعمارهم بين (٢٥ إلى ٣٥) سنة أيضا.أظهرت النتائج ارتفاعا معنويا في فعالية السوبر اوكسيد دسميوتيز وانخفاضا معنويا في فعالية الكاتليز لدى المصابين مقارنة بالأصحاء حيث بلغت قيمة (P-value) (٠,٠٠١ و ٠,٠٠٠) على التوالي ، بينت الدراسة ارتفاعا معنويا في مستوى الفوسفاتيز القاعدي لدى المصابين مقارنة بالأصحاء (P-value = 0.00). تم الاستنتاج بان اختلاف الجنس ليس له علاقة بتغيير كلا من السوبر اوكسيد دسميوتيز،الكاتليز و الفوسفاتيز القاعدي.

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks the joints producing an inflammatory synovitis that often progresses to destruction of the articular cartilage and ankylosis of the joints, rheumatoid arthritis can also produce diffuse inflammation in the lungs, pericardium, pleura, sclera, and also nodular lesions, most common in subcutaneous tissue under the skin⁽¹⁻²⁾. Rheumatoid arthritis is a form of autoimmunity, the causes of which are still incompletely known, it is a systemic (whole body) disorder principally affecting synovial tissues⁽³⁾ (Fig.1).

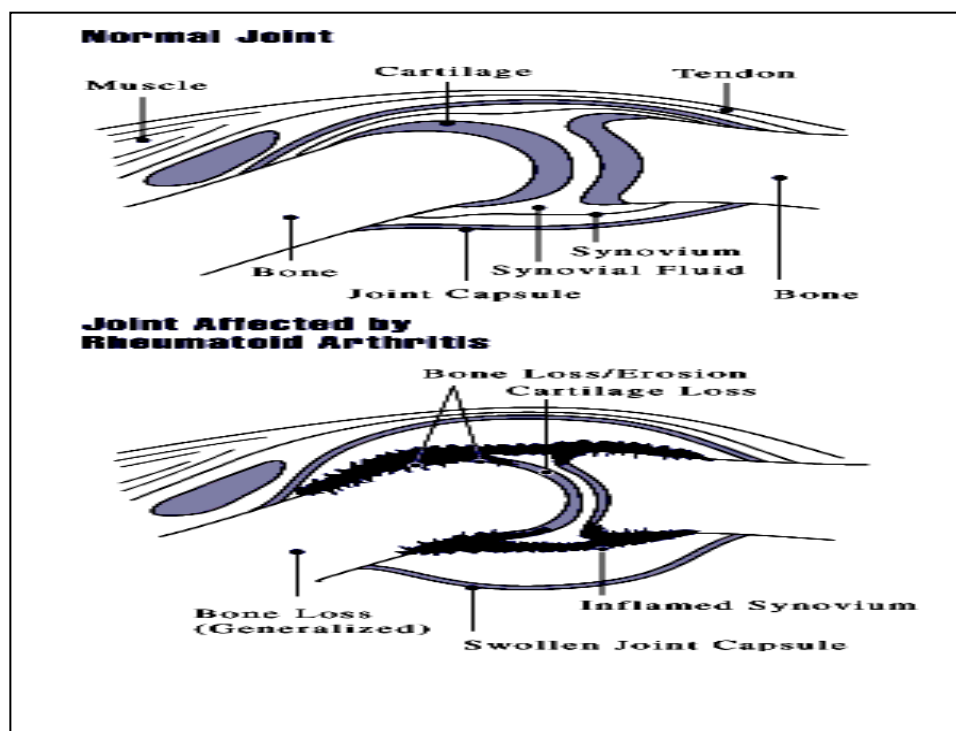
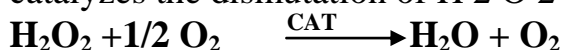


Fig. (1) A diagram showing how rheumatoid arthritis affects a joint

In recent years a great number of studies have investigated the possible role of reactive oxygen species(ROS) in the aetiology and pathogenesis of rheumatoid arthritis (RA) ⁽⁴⁾. Increased oxidative stress as a result of increased free radical formation has been suggested as a contributor to vascular damage , reactive oxygen and nitrogen species (RONS) such as superoxide ($O_2^{\cdot-}$),hydrogen peroxide (H_2O_2) hydroxyl radicals ($\cdot OH$) and peroxynitrite ($ONOO^{\cdot}$), have been ascribed an important role in oxidative stress contributing to the progression of inflammatory diseases ⁽⁵⁾.A number of protective enzymes exist for the regulation of RONS, these include superoxide dismutase (SOD) and catalase are prevent oxidative damage by $O_2^{\cdot-}$, the body developed an antioxidant defence enzyme, specific for $O_2^{\cdot-}$, called superoxide dismutase(SOD).⁽⁶⁾ The SOD catalyses the dismutation of $O_2^{\cdot-}$ to H_2O_2 and O_2 via an alternate oxidation and reduction reaction. ⁽⁷⁾



The H_2O_2 formed by SOD and other processes is scavenged by catalase, that catalyzes the dismutation of H_2O_2 into water and molecular oxygen⁽⁸⁾.



Catalase is important regulators of oxidative stress and inflammation, and may contribute to the development of rheumatoid arthritis (RA), free radicals change the permeability of cell membranes, thus causing inflammation and impairment of the functions of organs antioxidant enzyme catalase is responsible for detoxification of H_2O_2 and prevent oxidative damage.⁽⁹⁾ Several authors have considered that disease activity is related to the level of alkaline phosphate (ALP), therefore rises in this enzyme are considered to be attributable to(RA) itself .⁽¹⁰⁾ Alkaline phosphatase (ALP) is an enzyme found in all tissues, with particularly high concentrations of

ALP observed in the liver, bile ducts, placenta, and bone.⁽¹¹⁾ Alkaline phosphatase is an ortho phosphoric monoester phosphohydrolase catalyzing the hydrolysis of organic esters at alkaline pH, indicating that alkaline phosphatase is involved in fundamental biological processes⁽¹²⁾.

Material and Methods

Determination of superoxide dismutase(SOD)

To 50 μ L of serum, 75mM of tris-HCL buffer, 30 mM of EDTA and 2mM of pyrogallol were added. An increase in absorbance was recorded at 420nm for 30 min. The activity of SOD is expressed as U/ml of serum⁽¹³⁾.

Determination of catalase activity (CAT)

Catalase was assayed calorimetrically at 620 nm and expressed as μ moles of H₂O₂ consumed min/ml of serum described by Sinha⁽¹⁴⁾. The reaction contain 1.0ml of 0.01M phosphate buffer with 0.1ml of serum and 0.4ml of H₂O₂, the reaction was stopped by the addition of 2ml of dichromate acetic acid reagent.

Determination of alkaline phosphatase activity(ALP)

Alkaline phosphatase activity is color metrically determined according to the following methods . Phenol is measured in the presence of amino anti-pyrene and potassium ferricyanide ,the presence of sodium arsenate in the reagent to stop the enzymatic reaction for the more of color change was estimated at 510 nm.⁽¹⁵⁾

Results and Dissuasion

A significant increase between Rheumatoid arthritis (RA) patients and healthy control in superoxide activity show in table (1).

Table (1):Effect of superoxide dismutase(SOD) (U/ml) on Rheumatoid arthritis patients and healthy control.

Subject	Mean \pm SD	P-value	Sign.
Control	0.476 \pm 0.067	-----	-----
Patient	1.051 \pm 0.26	0.000	Sign.

The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress.⁽¹⁶⁾ The significant increased in rheumatoid arthritis patients compared to healthy control due to joint inflammation such as arthritic diseases, it can only be assumed that these harmful effects might be caused by an increased formation of H₂O₂.⁽¹⁷⁾ Every cell in the body uses SOD to combat damaging free radicals produced by normal cellular reactions. Superoxide is the most dangerous and prevalent free radical produced in the body because it requires three electrons to rebalance itself, SOD exerts this benefit in both the mitochondria and the cytoplasm of the cell, it helps to protect the integrity of the joints, and the immune system from damage as well as reduce oxidative damage to the cells of the respiratory system⁽¹⁸⁾.

The role of catalase in defending cells and tissues against oxidative stress has been studied extensively, decreases in catalase activity in patients with tumors is more

likely to be due to decreased enzyme synthesis rather than to catalase mutations⁽¹⁹⁾. The results obtained significantly decrease in rheumatoid arthritis patients compared to healthy control, hydrogen peroxide could not have been detoxified due to decreased activities of catalase because hydrogen peroxide possibly converted to hydroxyl radical by iron in patients with rheumatoid arthritis⁽²⁰⁾. A deficiency of catalase activity is noted in many tissues of the body, including the red blood cells, bone marrow, liver, and skin, only about half of the affected individuals have symptoms, which consist of recurrent infections causing painful sores on gums, tooth loss, oral gangrene, altered lipid, carbohydrate, homocysteine metabolism and the increased risk of diabetes mellitus and arteriosclerosis⁽²¹⁾.

Table (2): Catalase activity (Katal) in rheumatoid arthritis patients and healthy control.

Subject	Mean± SD	P-value	Sign.
Control	0.319 ±0.103	-----	-----
Patient	0.083± 0.107	0.001	Sign.

alkaline phosphatase activity of bone cells is increased bone formation in embryonic calvaria at concentrations that stimulate bone formation in vivo, this work obtained significantly increase in rheumatoid arthritis patients compared to healthy control due to serum ALP activity may have to increase in parallel with disease activity and bone resorption is accompanied by concomitant bone formation and arise in serum ALP⁽²²⁾.

Table (3): Alkaline phosphatase (ALP) levels (U/L) in rheumatoid arthritis patients compared with control.

Subject	Mean± SD	P-value	Sign.
Control	72.4 ± 14.3	-----	-----
Patient	103.1± 7.8	0.00	Sign.

The results shown no significant difference in SOD, CAT and ALP activity between sex ($P > 0.05$).

Table(4):Effect of sex on SOD,CAT and ALP levels in rheumatoid arthritis patients.

Sex	Numbers	SOD P-value	CAT P-value	ALP P-value	Sig.
Males	7				
Females	8	0.317	0.102	0.801	No Sig.

Conclusions

- 1- Superoxide dismutase(SOD) increased in rheumatoid arthritis patients.
- 2- Catalase activity(CAT) decreased in rheumatoid arthritis patients compared with healthy control.
- 3- Alkaline phosphatase (ALP) levels increased in rheumatoid arthritis patients compared with healthy control.
- 4- There is no relationship between difference sex and disease.

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