The Relationship Between Free Radicals and Enzymatic Antioxidants In serum of Hemorrhoids Patients

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Abstract.

The antioxidant enzymes, Catalase (CAT) and superoxide dismutase (SOD) activate were investigation in serum of hemorrhoids patients(20 males and 20 females), mean ± SD aged 37±10.9 and 40±8.4 years respectively compared with (20 males and 20 females) healthy whose mean ± SD aged 31.4±11.6 and 35.6± 6.9 years respectively, were selected as the controls group.

These data suggest that hemorrhoids induces oxidative stress in serum of patients(male and females) compared with healthy controls and that antioxidants which illustrated in this study are effective against oxidative injury.

الخلاصة.

تم في هذا البحث قياس فعالية مضادات الأكسدة الإنزيمية الكاتليز CAT, والسوبر اوكسيد دسميوتيزSOD وعلاقتها مع الجذور الحرة المتولدة في مصل مرضى البواسير الشرجية, حيث تضمنت الدراسة 20 مريضا ذكرا و20 أنثى يتراوح معدل أعمارهم ± الانحراف المعياري بين37± 10.9و 40±8.4 سنه على التوالي, مقارنة بمجموعة الأصحاء المتكونة من 20 ذكر و 20 أنثى معدل اعمارهم 31±11.6 و 35.6± 6.9 سنه على التوالي. أوضحت النتائج إن هناك ارتفاعا معنويا في مستوى الأنزيمات التي تعمل كمضادات أكسدة نتيجة لزيادة إنتاج الجذور الحرة المتولدة بتأثير مرض البواسير الشرجية والتي قد تعتبر كمؤشر لتقدير الإجهاد التأكسدي لدى مرضى البواسير الشرجية

Introduction.

Hemorrhoids diseases are similar to those of more serious anorectal pathologies, particularly, carcinoma and inflammatory bowel disease[(1)]. Hemorrhoids are divided into internal and external Hemorrhoids and the internal variety is further sub divided into four stages, based principally on the degree of the prolapse. Closer evaluation of the rectum for hemorrhoids requires an exam with an anoscope, a hollow, lighted tube useful for viewing internal hemorrhoids, or a proctoscope, useful for more completely examining the entire rectum [(2)].

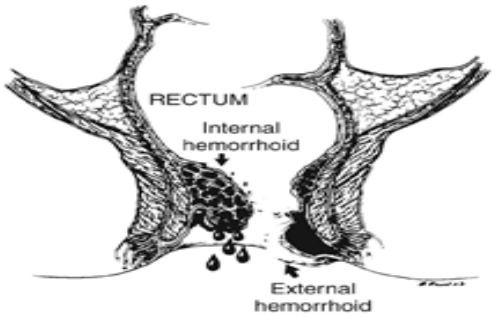

Fig.(1):- Internal and external hemorrhoids[2].

First degree Hemorrhoids are pathogenically enlarged but never prolapsed, remaining un their normal anatomical position in the anal canal, they may be asymptomatic, but even when they produce symptom, they can be seen only with proctoscope. Second degree Hemorrhoids cannot be seen on external examination, but the patients give history of the prolapse with defecation upon proctoscope, they bulge prominently, and the site of bleeding is often obvious. In third degree Hemorrhoids, the prolapse occurs with every bowel motion, occasionally with straining and exertion specially when standing, while the fourth degree of Hemorrhoids are permanently prolapsed and thus prone to thrombosis, they are painful and often bleed profusely, the over lying mucosa often becomes keratinized[3].

The most important free radicals in many disease states are oxygen derivatives, particularly superoxide($O_2^{-}\bullet$), the hydroxyl radical($OH^{\bullet}$), peroxyl($LOO^{\bullet}$) and peroxynitrite ($ONOO^{-}$), oxidative stress is the term referring to the imbalance between the generation of reactive oxygen species(ROS) and the activity of antioxidant defenses[4]. Nitric oxide radical has been identified as a biologically important molecule involved in a number of physiological processes.[5, 6].

The superoxide dismutases catalyse the dismutation of superoxide to hydrogen peroxide.

$$O_2^{-}\bullet + O_2^{-}\bullet + 2H^{+} \longrightarrow H_2O_2 + O_2$$

The hydrogen peroxide must then be removed by catalase or glutathione peroxidase. There are three forms of superoxide dismutase in mammalian tissues, each with a specific subcellular location and different tissue distribution. Copper zinc superoxide dismutase (CuZnSOD): is found in the cytoplasm and organelles of virtually all mammalian cells[7] It has two protein subunits, each containing a catalytically active copper and zinc atom. Manganese superoxide dismutase (MnSOD): is found in the mitochondria of almost all cells and has consists of four protein subunits, each probably containing a single manganese atom.[8] Extracellular superoxide dismutase (EC-SOD): is a copper and zinc containing SOD distinct from the CuZnSOD described above. EC-SOD is synthesised by only a few cell types, including fibroblasts and endothelial cells, and is expressed on the cell surface where it is bound to heparan sulphates.[9]

Catalase was the first antioxidant enzyme to be characterized and catalyses the two stage conversion of hydrogen peroxide to water and oxygen:

$$catalase\text{–}Fe(III) + H_2O_2 \rightarrow compound\ I$$

$$compound\ I + H_2O_2 \rightarrow catalase\text{–}Fe(III) + 2H_2O + O_2$$

Catalase is largely located within cells in peroxisomes, which also contain most of the enzymes capable of generating hydrogen peroxide. The amount of catalase in cytoplasm and other subcellular compartments remains unclear, because peroxisomes

are easily ruptured during the manipulation of cells. The greatest activity is present in liver and erythrocytes but some catalase is found in all tissues[10].

Patients and Methods.

40 patients, 20 males and 20 females aged 37±10.9 and 40±8.4 years respectively, and 40 healthy subjects 20 males and 20 females aged 31.4±11.6 and 35.6± 6.9 years respectively, as a control group, blood samples were collected from patients with hemorrhoids of various degrees were collected. After clotting, serum was separated by centrifugation, the analytical determinations described below were either performed immediately, or serum was stored at -20°C and used within 72 hours.

Methods.

To 50 µL 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[11].

Catalase was assayed calorimetrically at 620nm and expressed as µmoles of H_2O_2 consumed \min\ml of serum described by Sinha[12]. The reaction contain 1.0 ml of 0.01M phosphate buffer with 0.1 ml of serum and 0.4 ml of H_2O_2. The reaction was stopped by the addition of 2 ml of dichromate acetic acid reagent.

Statistical analysis.

All results are expressed as a mean ± SD(standard deviation), comparison between patients and controls were preformed by the student's t- test. Person's correlations were used to determine relationship between parameters studied. A value of $p \geq 0.05$ was considered statistically significant.

Results and Discussion.

Many patients suffer from the complication of hemorrhoids that cannot be explained by a proctoscope. Reactive oxygen species (ROS) have been proposed to contribute to this complication. Consistent with this we found evidence for induction of the antioxidant for SOD and catalase in hemorrhoids, to determine whether increased antioxidant protection could reduce the complication after infection.

The SOD activity of males and females patients are significant increase (P=0.001, 0.00) respectively, compared with healthy controls, Fig(1). While the analysis showed no significant increase between males and females(P=0.07)

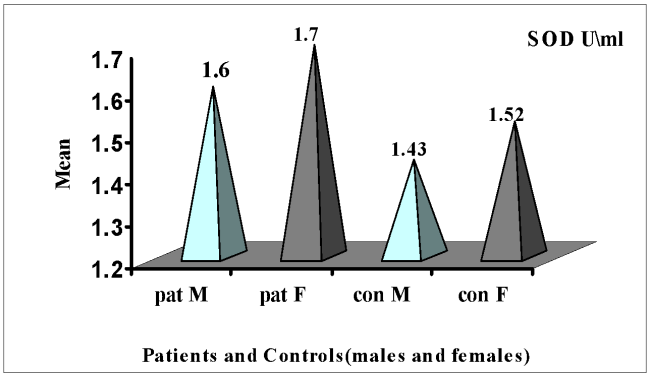

Fig.(1) The SOD activity in U\ml for patients and controls.

The CAT activity of males and females patients are significant increase (P=0.00, 0.00) respectively, compared with healthy controls, Fig(2). Beside a significant increase between males and females(P=0.05).

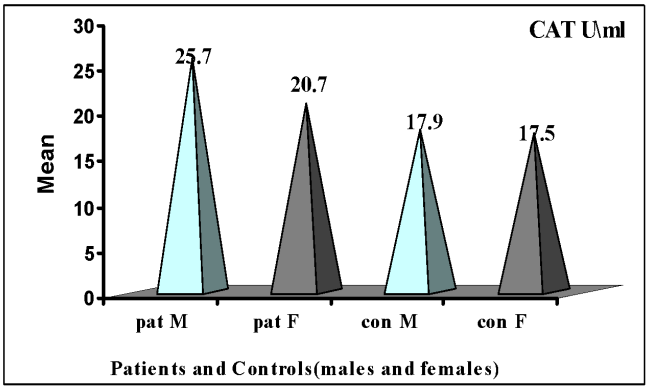

Fig.(2) The CAT activity in U\ml for patients and controls.

An increasing in activity of SOD and CAT in serum of hemorrhoidal patients are due to ability of SOD to convert harmful superoxide radicals into H_2O_2, which in turn is metabolized to harmless water and oxygen by catalase[13]. However, its most vital property is the ability to cross cell membranes freely, which superoxide generally cannot do[14]. Therefore, hydrogen peroxide formed in one location might diffuse a considerable distance before decomposing to yield the highly reactive hydroxyl radical, which is likely to mediate most of the toxic effects ascribed to hydrogen peroxide. Therefore, hydrogen peroxide acts as a conduit to transmit free radical induced damage across cell compartments and between cells.

Our results show that SOD and CAT protects against the complication of hemorrhoids.

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