

Catalase evaluation in different human diseases associated with oxidative stress

Abdul Salam A. Al-Abrash, MSc, Faizeh A. Al-Quobaili, PhD, Ghada N. Al-Akhras, PhD.

ABSTRACT

Objective: Catalase is an enzyme present in most of the aerobic cells, it protects them from oxidative stress by catalyzing the rapid decomposition of hydrogen peroxide (H₂O₂) in two types of reactions depending on its peroxidatic and catalatic activities. The aim of this study was to measure the erythrocytes catalase activity by a reliable method in normal subjects with different age categories, and patients whom suffer from different diseases associated with oxidative stress (inflammatory, tumor, diabetes, cardiovascular diseases, anemia and Wilson's disease).

Methods: Erythrocytes catalase activity was measured, by peroxidatic method (Johansson-Borg method), in 210 apparently healthy subjects, (117 males and 93 females). The range of their ages was from 7 months to 65 years, and in 454 patients their ages ranged from 3 months to 74 years, whom suffer from the above mentioned diseases which resulted in oxidative stress. The comparison had been made between the Johansson-Borg and the UV catalase methods.

Results: Strong correlation was found between the two methods, peroxidatic and catalatic ($r=0.99$, $P<0.0001$), but

the catalase solutions were unstable when the temperature was raised. The normal range of catalase was found to be 2869 ± 1039 u/g Hb. It was found that the catalase activity increased in the studied morbidity groups (eg. 188% in oxidative anemia). An accepted decrease 50% was noted in catalase activity when Vitamin E was administered to anemic patients suffering from oxidative stress.

Conclusion: There was an increase in catalase activity in all studied patients suffering from oxidative stress (cardiovascular diseases, diabetes, tumor, inflammation, dermatological diseases, anemia and Wilson's disease). The catalase activity was not affected by age, sex or the anticoagulant agent, which was used to collect the blood samples. It was found that the Vitamin E supplement decreased the catalase activity and improved the state of anemic oxidative stress patients.

Keywords: Catalase, oxidative stress, anemia, peroxidatic activity, catalatic activity, Vitamin E, red blood cells, cardiovascular disease and inflammation.

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Reactive oxygen species (ROS) are substances released during oxidative metabolism. The reactions of ROS with macromolecules can lead to DNA mutations, changes in the structure and function of proteins and peroxidative damage of cell membrane lipid.¹

Since free radicals have been implicated in the pathogenesis of a variety of human diseases, this has prompted interest in the evaluation of cellular levels of antioxidant agents.² Catalase (EC: 1.11.1.6) is an antioxidant enzyme with high specific activity present in all aerobic cells. In erythrocytes, catalase

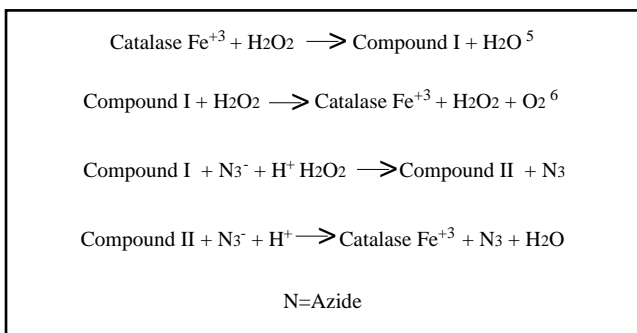
and glutathione peroxidase jointly protect hemoglobin from oxidative damage.²

Catalase catalyzes the rapid decomposition of hydrogen peroxide by two types of reactions. Both types include a first step formation of compound I, which consists of the enzyme and hydrogen peroxide. The catalatic activity catalyzes a reaction with a second molecule of hydrogen peroxide producing water and oxygen.^{3,4} The reaction can be described in most general terms by the following equations found in Table 1. The peroxidatic activity of the enzyme,

From the Department of Clinical Laboratory, (Al-Abrash), Department of Biochemistry & Microbiology, (Al-Quobaili & Al-Akhras), Faculty of Pharmacy, Damascus University, Syria.

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Address correspondence and reprint request to: Dr. Faizeh Al-Quobaili, PO Box 6735, Damascus, Syria. Fax. +963 11 661 1680/212 8278.

Table 1 - Equations that describe general reactions.

first described by Keilin and Hartree,⁷ catalyzes reactions of compound I with hydrogen donors other than hydrogen peroxide as aliphatic alcohols, formic acid, azide, phenols.^{2,4} The peroxidatic activity of catalase is most evident at relatively low concentration of hydrogen peroxide.³

Many methods for assaying catalase depend on the catalytic function of this enzyme; such methods involve measurements of either hydrogen peroxide consumption or oxygen production.^{2,3} In this study the peroxidatic function of catalase was utilized for determining its activity in erythrocytes.

Methods. Study groups. The catalase activity of erythrocyte and hemoglobin was determined in 664 subjects divided into 9 groups detailed as follows: the healthy subjects (93 females and 117 males); the patients suffering from the following

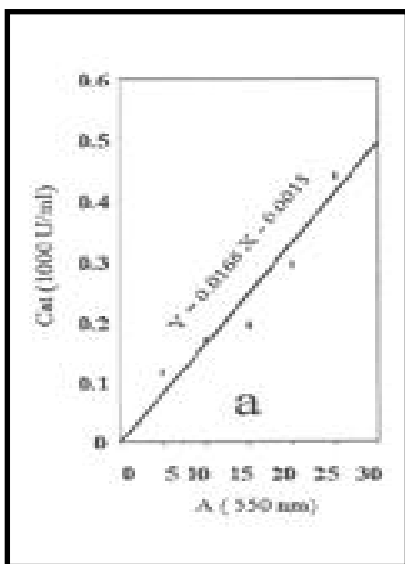
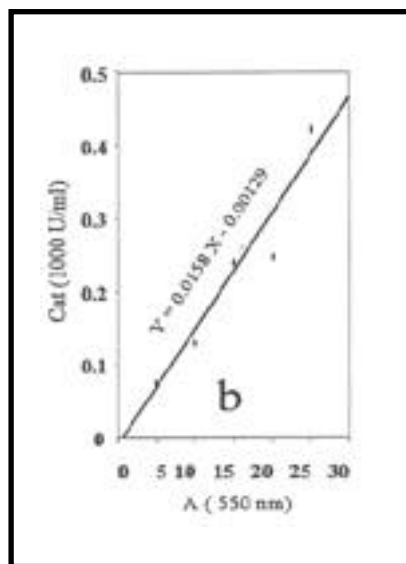
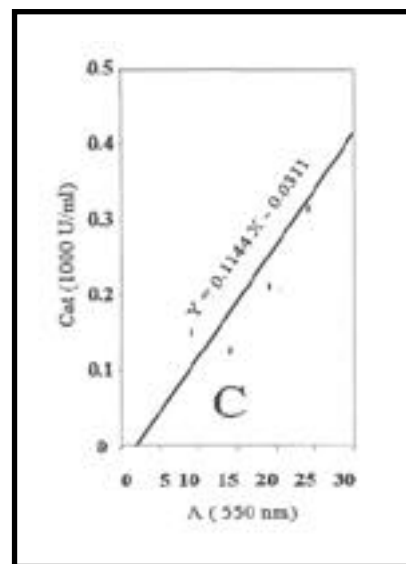
diseases, inflammatory and infectious (166 subjects), tumor (34 subjects), dermatological (20 subjects), diabetes (34 subjects), catheter and cardiovascular (61 subjects), no accompanied anemia with oxidative stress (patients whom have not blood transfusions or hemolysis) (42 subjects), Wilson's disease (2 subjects) and anemia accompanied with oxidative stress (95 subjects). Blood samples were collected into heparinized tubes (for catalase assay) and into EDTA tubes (for hemoglobin determination).

Materials. All reagents used were analytical grade (phosphate buffer, hydrogen peroxide, methanol, potassium hydroxide, potassium periodate, formaldehyde solution, hydrochloric acid, bovine liver purified catalase 2980 U/mg (Fluka Biochemika, Switzerland), purpald as a chromogen (Fluka Biochemika, Switzerland) and Hemoglobin electrophoresis kit (Helena, France). The apparatus used were Spectronic 601 (USA), Spectronic 101 (USA) and Jasco Uvidec-66 (Japan) as spectrophotometers. Orion for pH accuracy, ABBOTT Cell-Dyne 3500 (USA) as hematology analyzer.

Hemoglobin determination. Hemoglobin concentrations were quantified in ABBOTT Cell-Dyne 3500, using Drabkin method.⁸

Preparation of erythrocyte lysates. Blood was collected into heparinized tubes. After separation of plasma, hemolysates were prepared by adding 1 volume of blood to 19 volumes of ice-cold deionized water. The resulted hemolysates were diluted (1:500) with deionized water.

Erythrocytes catalase assay. The peroxidatic activity of catalase was determined by an assay based

**Figure 1a** - Changes in peroxidatic activity of catalase at 20°C.**Figure 1b** - Changes in peroxidatic activity of catalase at 25°C.**Figure 1c** - Changes in peroxidatic activity of catalase at 37°C.

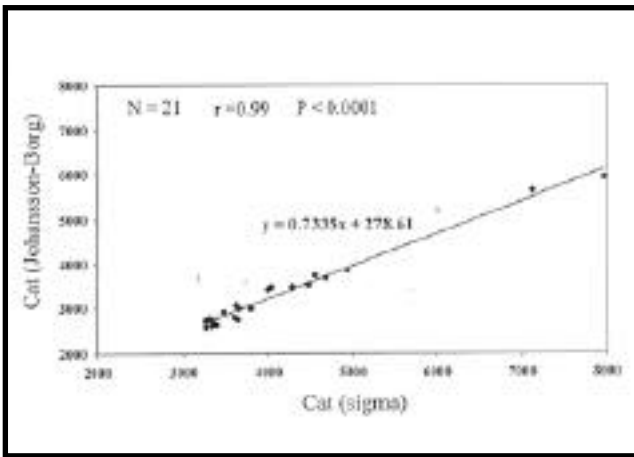


Figure 2 - Comparison between Johansson-Borg method and Sigma method.

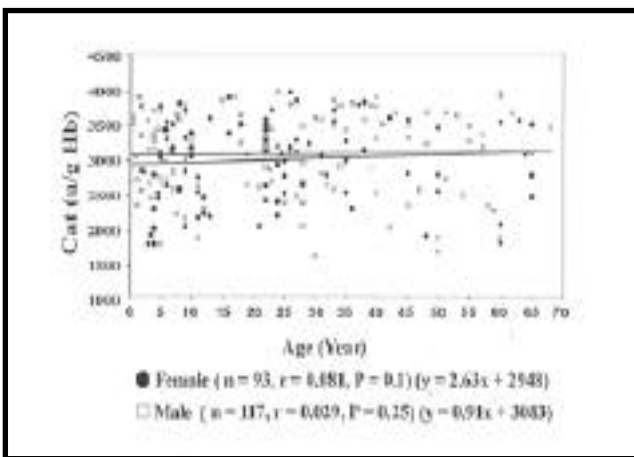


Figure 3 - Relation between catalase activity and age for males and females.

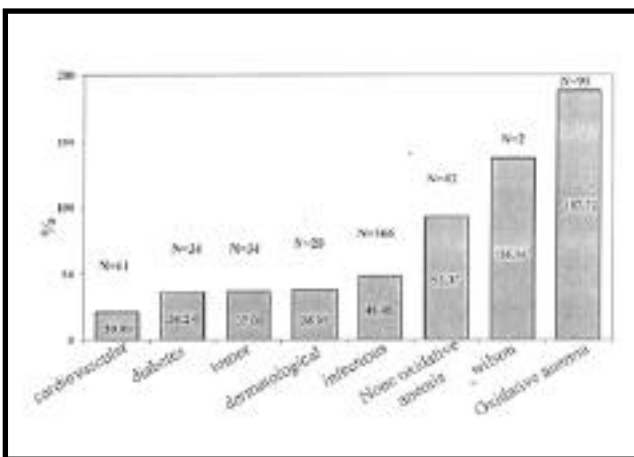


Figure 4 - Percentage of increased catalase activity in pathological groups.

on the method described by Johansson and Borg,^{2,3} which depends on the reaction of formaldehyde formed from methanol with purpald to produce a chromophore. Quantitation was carried out by measuring the absorbance at 550 nm in comparison with catalase calibrators as reference. This method was compared with the method mentioned by Sigma Company and described by Aebi.⁵ Depending on the catalytic function of catalase,⁹ by calculation of the time required to decrease the absorbance of hydrogen peroxide solution at 240 nm (A 240), after adding catalase contained sample, from 450 to 400, in the presence of standard series of catalase solution. One unit of catalase is equal to 1 μ mol of H_2O_2 decomposed/minute.

Results. The precision of Johansson-Borg method was assessed by repeated assay of pools of hemolysates (in daily series and from day to day). The results were acceptable and it was found that the storage of red blood cells (after washing with 0.9 g % NaCl) at $-20^\circ C$ was better than the storage of hemolysates at $-20^\circ C$. This may be due to the instability of catalase solutions in water. The colored product (Johansson-Borg method) was stable for at least 24 hours at $+4^\circ C$.

By screening peroxidatic activity of catalase at three different temperatures, it was noticed that the peroxidatic activity was decreased when temperature increased (Figure 1).

Comparing between Johansson-Borg method and the other method depends on catalytic function of catalase. A strong correlation was found between them $r = 0.99$ and $P < 0.0001$ (Figure 2).

No significant changes were noticed between the hemoglobin values of blood collected into heparin or EDTA ($r = 0.997$ and $P < 0.0001$), or when blood samples were stored for 48 hours at $+4^\circ C$, or on hemoglobin values, after 6 days of Vitamin C supplement (3 gms a day).

The reference range of catalase, assayed by Johansson-Borg method, (95% of all values) was 2869 ± 1039 U/g Hb in the healthy individuals group (210 subjects).

No significant difference was found in the catalase activity between the blood samples collected into heparin or EDTA ($r = 0.992$ and $P < 0.0001$). Catalase activity has not been affected by age ($P = 0.1$ and $r = 0.0254$) or sex ($P = 0.1$) (Figure 3). The study revealed a strong correlation between the increased catalase activity and cardiovascular diseases ($P < 0.0001$). The augmentation was about 21%. There was also an augmentation in catalase activity in diabetes patients, about 36% ($P < 0.0001$), about 37% ($P < 0.0001$) in tumor patients, about 38% ($P < 0.0001$) in dermatological diseases, about 49% ($P < 0.0001$) in inflammatory and infectious diseases and about 93% in patients suffering from anemia non accompanied with oxidative stress ($P < 0.0001$). While

in Wilson's disease, the augmentation was about 137% ($P < 0.0001$). The activity of catalase increased to about 188% ($P < 0.0001$) in the patients suffering from anemia accompanied with oxidative stress (whom have transition iron ions resulted from iron overload by blood transfusions and hemolysis or both) (Figure 4).

No significant change on catalase activity was found in 20 healthy subjects after they had been supplied with Vitamin C (3 g/day).

When comparison was made between 58 patients (suffering from oxidative anemia and using desferal) and 13 patients (suffering from oxidative anemia who do not use desferal), no significant changes in catalase activity were found.

An acceptable decrease in catalase activity was noticed in 50% of patients suffering from anemia accompanied with oxidative stress whom they received a supply of Vitamin E (5mg/kg body weight/day for one month).

Discussion. Catalase is an enzyme exerting a dual function; it catalyzes the decomposition of hydrogen peroxide to produce water and oxygen (catalatic function) or oxidation of H donors (peroxidatic function). In our study the catalase activity was assayed in RBC utilizing peroxidatic function (Johansson-Borg method).

The colored product, formed (Johansson-Borg method), was stable for at least 24 hours at +4°C while Johansson 1988³ had referred, that the colored product was stable for at least 3 hours at room temperature. The un-stability of aqueous catalase solutions found was similar to Johansson 1988³ findings and the optimum pH found for the reaction was exactly 7.

There was a strong correlation between catalatic and peroxidatic functions of catalase (comparison between Johansson-Borg method and the method mentioned by Sigma Company) resembling Kisadere 1997² findings.

The reference range found (2869 ± 1039 U/g Hb) was slightly higher than that (1843 ± 250 U/g Hb) of Pastor 1998 findings in Spain.¹ This difference may be attributed to higher substrate concentration, which was used and to hereditary factors.

Sex or age has not affected catalase activity, similarly to Andersen 1997 findings in Denmark.⁶ Pastor 1998¹ had revealed that catalase activity had not been affected by age, while King 1997¹⁰ in (USA) had showed an increase in the catalase activity by age.

Casado study 1998¹¹ in Spain had showed a decrease in the catalase activity by age, and an increased activity in healthy women versus healthy men. In Hungary, the Vitai and Goth study in 1997 showed that the activity was lower in healthy women in comparison with men, while the ratios of blood

catalase activity to blood hemoglobin concentration were not different, and the decreased activity by age was evident in men more than in women. Also that study referred to hypocalasemia of inheritance causes. There was no correlation between catalase activity and anticoagulant agents (EDTA or heparin), used to obtain blood samples, similarly to Andersen 1997 finding.⁶ When 20 healthy subjects were supplied with Vitamin C (3g/day) for 6 days, no significant change appeared on catalase activity or hemoglobin concentrations, although Hoffman study 1991¹ referred to the role of Vitamin C in iron absorption, but most of the iron in food is non hemic.

The non effective role of Vitamin C found on catalase activity in erythrocytes may be attributed to the weak solubility of Vitamin C in lipids, so it is very difficult to influx across red cell membrane, and 6 days supplement of Vitamin C may be not sufficient to affect catalase activity.

Our study revealed that the Vitamin E supplement for 21 anemic patients, suffer from oxidative stress (5 mg/kg weight of body for one month) caused a decrease in catalase activity in 50% of these patients. This may be attributed to non sufficient doses or a non sufficient period of therapy. Malabsorption of lipid soluble vitamins in thalassemic patients, as Giardini in 1985¹² referred, may be the most importance reason.

By comparing between the catalase activity in patients suffering from anemia accompanied with oxidative stress, who received desferal and who did not receive it, no significant changes appeared. Since desferal is used when serum ferritin is more than 1000 mg/dl, the appreciated values between the two groups revealed the role of desferal in relieving the oxidative stress caused by transition iron ions.

All diseases which oxidative stress participates in were accompanied with an increase in catalase activity (cardiovascular diseases, diabetes, tumors, infections and inflammations, dermatological diseases, anemia, Wilson's disease). However, Casado's study¹¹ declared that catalase activity decreased in cardiovascular diseases. Oxidative stress in Wilson's patients may be attributed to transition iron (hemolysis) and copper ions, as Andreoli⁷ referred.

Hemoglobin concentration has not been affected by anticoagulant agents (EDTA or heparin) nor by storage of whole blood at +4°C for 2 days, similarly to Powers 1989 findings.⁸

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