DOI: HTTP://DOI.ORG/10.31925/FARMACIA.2018.6.9 **ORIGINAL ARTICLE**

# THE CAT AND SOD ACTIVITIES IN PATIENTS WITH CHRONIC SUPPURATIVE OTITIS MEDIA

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**Abstract**

Oxidative stress plays an important role in multiple diseases by disturbing the oxidant-antioxidant balance. The aim of this study was to evaluate the antioxidative status in patients with chronic suppurative otitis media (CSOM). We conducted a prospective study that included 60 patients diagnosed with chronic suppurative otitis media with and without cholesteatoma. The catalase (CAT) and superoxide dismutase (SOD) activities were determined in erythrocytes respectively serum of the patients. We noticed that the antioxidant enzyme SOD activity in the patients with CSOM with and without cholesteatoma had statistically significant lower values compared to the healthy subjects ($p < 0.05$). For the same groups the CAT activity was statistically significant increased ($p < 0.05$) as compared to the healthy group. As a conclusion, oxidative stress seems to be involved in the pathogenesis of CSOM with and without cholesteatoma, leading to chronic infections and tissue injury.

**Rezumat**

Stresul oxidativ joacă un rol important în multiple boli prin modificarea balanței prooxidant-antioxidant. Scopul acestui studiu a fost evaluarea statusului antioxidant la pacienții cu otita medie supurativă cronică (OMCS). Am efectuat un studiu prospectiv care a inclus 60 de pacienți diagnosticați cu otită medie cronică supurată cu și fără colesteatom. Activitățile catalazei (CAT) și superoxid dismutazei (SOD) au fost determinate la nivel eritrocitar și respectiv seric. S-a observat că activitatea enzimei antioxidante SOD la pacienții cu OMCS cu și fără colesteatom a avut valori semnificativ statistic mai scăzute comparativ cu grupul de subiecți sănătoși ($p < 0,05$). În cazul CAT s-a înregistrat o creștere semnificativ statistică a activității enzimatice ($p < 0,05$). În concluzie, stresul oxidativ poate fi implicat în patogeneza OMCS cu și fără colesteatom, favorizând cronicizarea infecției și leziuni tisulare.

**Keywords**: cholesteatoma, oxidative stress, chronic otitis media, catalase

## Introduction

Chronic suppurative otitis media (CSOM) is a local inflammatory process, involving hearing loss and purulent discharge [15, 19]. A lot of factors like inflammatory mediators (proteins, peptides, glycol-proteins, cytokines, oxygen free radicals), local factors (Eustachian tube dysfunction, malformations, tympanic membrane perforation) maintain inflammation and disease progression [14].

The implications of oxidative stress have not been fully explored in CSOM pathogenesis. In the literature there are only a few articles that explore this topic [5, 6, 9, 10].

Oxidative stress was first described in 1985 and represents the imbalance between oxidants and antioxidants, in favour of oxidants, with pathogenic potential [16, 20]. Oxidative stress is generated by overproduction of free radicals representing molecules containing one or more electrons. Free radicals are represented by reactive oxygen (superoxide, hydroxyl, peroxyl, hydroxypiperyl), nitrogen, and sulphur species. Antioxidant defence is achieved through endogenous or exogenous antioxidants. The

antioxidants (chemical or biological) have the role of neutralizing the reactive species of oxygen, nitrogen, and peroxidation products.

During the inflammatory process of the middle ear and mastoid, superoxide radicals are generated to increase bactericidal activity leading to free radical growth followed by the alteration of oxidative balance [21]. The cells can be protected from the reactive species attack through enzymatic anti-oxidants: superoxide dismutase, catalase, lactoperoxidase, glutathione peroxidase and antioxidant molecules: ascorbic acid, tocopherol, uric acid, albumin, creatinine, bilirubin, glutathione.

## Materials and Methods

The study was performed between 2017 and 2018 in the Clinical Rehabilitation Hospital from Iaşi, Romania. A group of 60 patients was assessed, aged between 9 and 58 years, 28 with CSOM without cholesteatoma (12 from the rural area, 16 from the urban area, 13 females and 15 males) and 21 patients with CSOM with cholesteatoma (12 from rural area, 9 from urban area, 11 females, 10 males) and 11 with cholesteatoma recidivism (7 from rural area, 4 from urban area, 6 females, 5 males). The inclusion criteria were: clinical and imagistic diagnosis of CSOM with or without cholesteatoma, patient without any other known acute or chronic pathologies, agreement to participate in the study. Exclusion criteria were represented by other chronic or acute pathology other than ear disease, history of smoking, under medication, including vitamins. All patients were given written informed consent.

The control group included 30 healthy patients, 13 from rural and 17 from urban areas, aged between 6 and 54 years old with no chronic and acute diseases, medication administration or history of smoking.

Blood samples were taken after 12 hours of fasting collected into empty tubes and immediately stored on ice at 4□. The serum was separated from the cells by centrifugation at 3.000 rpm for 10 minutes. Serum samples used for the measurement of super-oxide dismutase (SOD) levels were stored at –20□ until they were used. In the erythrocyte lysate (obtained accordingly to the supplier's protocol; 0.2 mL erythrocytes was homogenized with 0.2 mL Assay Buffer, then centrifuged at 10.000 rpm for 15 min. at 4□ and the supernatant was stored at –80□) catalase (CAT) activity was determined .

*Determination of SOD*

SOD, an important antioxidant enzyme, was assessed using SOD Assay Kit-WST for research use, which allows SOD determination by using Dojindo's water-soluble tetrazolium salt to produce a water-soluble formazan pigment over reduction with a superoxide anion. The reduction rate with $O_2$ is dependent to the to the xanthine oxidase activity, and can be inhibited by SOD.

*Determination of CAT*

CAT was measured using a CAT Assay Kit (Abnova) for research use, according to the supplier protocol. Initially CAT reacts with hydrogen peroxide to produce water and oxygen. After that, the remaining unconverted hydrogen peroxide reacts with OxiRed probe to produce a new product, detected at 570 nm. CAT activity is in inverse proportional relation to the absorbance.

The patients were divided into 4 groups: group M which represent healthy subjects, group C which includes patients diagnosed with CSOM with cholesteatoma, group R includes patients with cholesteatoma recidivism and group O which represents patients diagnosed with CSOM without cholesteatoma.

*Statistical analysis* was performed using SPSS program version 18.0.

## Results and Discussion

The oxidative stress status is indicated by the increase of reactive species (oxygen superoxide and hydrogen peroxide). The measurement of the concentration of reactive species was performed indirectly by measuring the activity of antioxidant mobilization systems following the generation of recurrent species. Therefore, the increase in antioxidant enzyme activity indicates reactive species that cause an oxidative imbalance.

Reactive oxygen species induce many cellular processes like cell proliferation and uncontrolled cell growth which may cause tumours and inflammation development. In the case of chronic ear inflammation, the production of reactive oxygen species may increase the dysregulation processes and development of preneoplastic condition [2, 16, 17].

The Skewness/Kurtosis tests ($-2 < p < 2$) suggest that the SOD and CAT series values were homogeneous.

The SOD activity (inhibition rate %) in the studied groups varied: between 13.744 and 37.915 in the group with cholesteatoma recidivism, between 20.853 and 45.972 in the group with CSOM with cholesteatoma, and between 24.562 and 66.173 in the group with CSOM without cholesteatoma. In the healthy subjects group, enzymes activities values ranged between 31.006 and 88.408 (Table I).

**Table I**

Statistical description of study groups

| Parameter | | Group M | Group R | Group C | Group O |
|---|---|---|---|---|---|
| Number of subjects/patients | | 30 | 11 | 21 | 28 |
| Mean | | 64.475 | 22.929 [a)] | 32.541 [a) b)] | 36.316 [a) a) ns)] |
| Median | | 64.723 | 18.276 | 36.019 | 32.123 |
| Standard deviation | | 12.818 | 9.290 | 7.639 | 10.129 |
| Variance | | 19.882 | 18.312 | 28.349 | 12.589 |
| Skewness Test | | -0.351 | 0.769 | -0.032 | 1.938 |
| Std. Error of Skewness | | 0.427 | 0.661 | 0.501 | 0.441 |
| Kurtosis | | 0.756 | -1.081 | -0.726 | 1.916 |
| Std. Error of Kurtosis | | 0.833 | 1.279 | 0.972 | 0.858 |
| Minim | | 31.006 | 13.744 | 20.853 | 24.562 |
| Maxim | | 88.408 | 37.915 | 45.972 | 66.173 |
| Percentiles | 25 | 59.027 | 16.256 | 26.094 | 30.191 |
| | 50 | 64.723 | 18.276 | 36.019 | 32.123 |
| | 75 | 71.549 | 30.028 | 36.967 | 39.922 |

a) $p < 0.001$, b) $p < 0.05$, ns) $p > 0.05$

The mean level of SOD activity in patients with cholesteatoma recidivism, followed by patients with CSOM with cholesteatoma, was found to be significantly lower compared to the control group (22.929; 32.541 (p = 0.004) *vs* 64.475; p = 0.001). The mean level in patients with CSOM with cholesteatoma was slightly lower than compared to CSOM without cholesteatoma, but both were significantly lower compared to healthy group (32.541; 36.309 (p = 0.160) *vs.* 64.475, (p = 0.001)) (Figure 1).

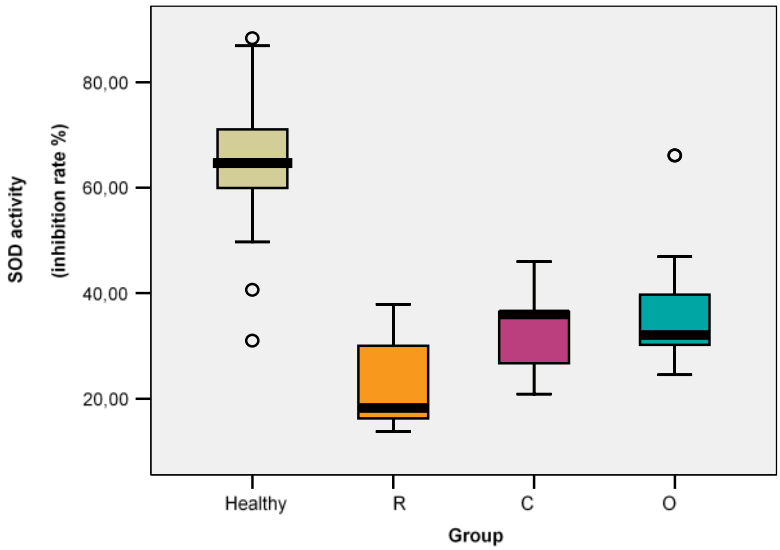


**Figure 1.**

Mean levels variations of SOD activity in healthy subjects group (M), patients with cholesteatoma recidivism (R), CSOM with cholesteatoma (C), CSOM without cholesteatoma (O)

CAT activity ranged between: 1.216 mU/mL and 1.910 mU/mL in the group of patients with cholesteatoma recidivism, 1.253 mU/mL and 2.531 mU/mL in patients with CSOM with cholesteatoma, 0.611 mU/mL and 2.461 mU/mL in patients with CSOM without cholesteatoma. In the healthy subjects group, this enzyme activity ranged between 0.334 mU/mL and 1.289 mU/mL (Figure 2). One unit of CAT is the quantity of catalase that decomposes 1.0 µmol $H_2O_2$ *per* minute when the pH is 4.5 at 25ºC.

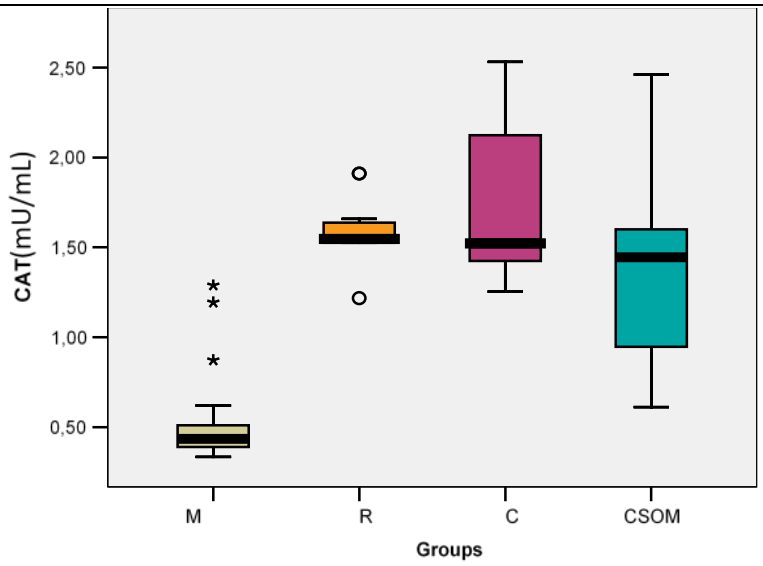


**Figure 2.**
Mean levels variation of CAT activity in the healthy subjects group (M), patients with cholesteatoma recidivism (R), CSOM with cholesteatoma (C), CSOM without cholesteatoma (O)

SOD is in the first line defence strategy against oxidative stress occurrence and progress. Thus, SOD is an essential antioxidant enzyme which detoxifies the superoxide anion generated by activated neutrophils and macrophages, by converting it to hydrogen peroxide [4, 8]. A low activity of SOD indicates a decrease of the antioxidant capacity that has been overcome by the hyper production of reactive oxygen species, thereby diminishing its ability to recover.

Yilmaz *et al.* found low levels of serum SOD and glutathione peroxidase in children with otitis media with effusion and showed that ventilation tubes insertion and adenoidectomy decreased the oxidative stress [22].

In the present study SOD values were significantly lower compared to the healthy group, similar results being obtained by Garca *et al.* [10]. The lowest values were recorded in patients with cholesteatoma recidivism. A chronic inflammatory process can be a trigger in cholesteatoma recidivism.

CAT acts in the decomposition of hydrogen peroxide which is a powerful oxidative agent, with toxic effects for cells. The catalase, present in the peroxisomes of all aerobic cells, has a detoxifying role, by protecting the cell from the toxic effects of hydrogen peroxide [7, 11].

Aktan B *et al.* found no change in erythrocytes CAT activity in guinea pigs with otitis media with effusion [3]. An increased CAT activity was observed in patients with chronic otitis media with tympanoclerosis compared to those without tympanosclerosis [13]. Garca *et al.* found in patients with CSOM lower CAT values compared to the healthy subjects group [10].

In the present study we found a statistically significant increase in CAT activity in patients with CSOM with and without cholesteatoma, compared to the healthy group. The increased activity of CAT could be attributed to a feedback result of hydrogen peroxide molecules on mRNA expression [12, 18]. There are studies that have shown the increase in catalase activity in all patients suffering from oxidative stress and not affected by age or gender [1].

## Conclusions

SOD activity registered statistically significant lower values in patients with CSOM with and without cholesteatoma and cholesteatoma recidivism, and CAT activity has statistically significantly increased, compared to the healthy group.

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