Assessment of Malondialdehyde and Ascorbic Acid Serum Levels in Group of Egyptian Children with Chronic Tonsillitis Before and After Tonsillectomy

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Abstract: Chronic tonsillitis (CT) with or without adenoid hypertrophy (AH) is a very frequently encountered disease in children. Despite the frequency of this disease and the significant morbidity it causes, its pathogenesis is not exactly known. The oxidation products are produced during inflammation and are involved in the tissue injury due to this inflammation. The antioxidants play role in neutralizing the destruction by these oxidation products. Low antioxidant levels in blood may predispose children to frequent upper respiratory infections by negatively influencing their immune system. ***Objective:*** To investigate the potential role of oxidants and antioxidants in the pathogenesis of CT in children. ***Patients and Methods:*** This case-control study was carried out on 15 children with CT who were planned to undergo tonsillectomy. The control group was made up of 15 age- and sex-matched healthy children with normal ENT examination. The blood levels of antioxidants (ascorbic acid) and peroxidation products {malondialdehyde (MDA)} were determined before and 1 month after the operation in the patients group and once only in the control group. **Results:** The preoperative serum level of MDA in patients was significantly higher than in controls (P-value < 0.05). While there was no statistically significant difference between its level in the patients after the operation and its level in the controls (P-value = 0.126). The serum levels of ascorbic acid in the patients pre and post tonsillectomy were significantly different when compared to controls (P-value < 0.05). In the patients group, the blood antioxidant level increased and oxidant level decreased significantly after the operation (P< 0.05). These levels after the operation never reached those of the control group. ***Conclusions:*** Oxidants and antioxidants played a significant role in the pathogenesis of chronic tonsillitis in children. These children are under significant oxidative stress. Tonsillectomy significantly decreased the oxidative stress in these patients, but could not normalize it completely.

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1. Introduction

The tonsils and adenoid are lymphoid tissues situated at the opening of the pharynx to provide primary defense against foreign matter(1).

Chronic tonsillitis (CT) with or without adenoid hypertrophy (AH) is a very frequently encountered disease in children. It leads to frequent absenteeism from school. The nutrition, growth, development and social lives of children are influenced negatively by this disease. Antibiotics and various adjunctive drugs used to treat this disease lead to many side effects in children. The economic burden of medical and surgical treatment of this disease is tremendous(2).

The clinical association between CT and subsequent appearance of rheumatic fever was recognized by many physicians... The possibility that acute rheumatic fever was one of the manifestations of infection with hemolytic streptococci, so each repeated episode of streptococcal pharyngotonsillitis increases the risk of rheumatic fever(3).

Lipid peroxidation has been implicated in various diseases and aging, including atherosclerosis, cataract, rheumatoid arthritis and neurodegenerative disorders. The free radical-mediated peroxidation of lipids has received a great deal of attention in connection with oxidative stress *in vivo*. (4)

A free radical can be defined as any species containing one or more unpaired electrons. Since these molecules are highly reactive they can cause tissue damage especially in cell membranes by reacting with cellular lipids, proteins, nucleotides and carbohydrates. (5)

The potential damaging effects of these free radicals are limited by a number of antioxidants in body. In addition to the antioxidant enzymes, namely catalase, superoxide dismutase, glutathione peroxidase and -6-phosphate dehydrogenase, the blood and some other tissues contain non-enzymatic antioxidants, namely α-tocopherol, β-carotene, retinol and ascorbic acid, among others. (2)

Low antioxidant levels in blood may predispose children to frequent upper respiratory infections by negatively influencing their immune system. (6)

Aim Of The Work:

The aim of this study was to investigate the potential role of oxidant (Malondialdehyde (MDA)) and antioxidant (Ascorbic acid) in the pathogenesis of CT in children aiming to be a promising target for preventing recurrent tonsillitis and preserving the tonsils.

This **2.Materials and Methods:**

Patients and controls:

Case-control study was carried out on 15 children with CT who were planned to undergo tonsillectomy in the period from March to May 2012.The operation was done under general anesthesia in Otorhinolaryngology department in Al Zahra University hospital.

The patients included in the study were from 4 to 7 years old children. CT was diagnosed by history and ENT examination.

Frequent attacks of tonsillitis, respiratory obstruction by enlarged tonsils, chronically inflamed tonsils with white debris coming from their crypts, congestion of anterior and posterior billers and enlarged jugulodigastric lymph nodes were accepted as indications for tonsillectomy.

Exclusion criteria:

1. Chronic infection other than CT e.g., TB.
2. Chronic inflammation e.g., Cystic fibrosis.
3. Systemic diseases.
4. History of operation to the tonsils and adenoids.

The control group was made up of 15 age- and sex-matched apparently healthy children, as confirmed by history, ENT examination and basic laboratory analysis.

Written informed consent was obtained from the parents of all children who participated in this study.

Laboratory investigations:

The patients were subjected to the following:

- The routine pre operative blood tests (CBC, ESR, PT, ASOT and CRP).
- 5 cc of venous blood was obtained before the operation.
- Another blood specimen was obtained from the patients group one month after the operation.

Blood was obtained from the control group just once.

The serum was separated from all the blood samples of patients and controls and used to assess the following:

a) MDA level as a peroxidation product (lipid peroxide) by colorimetric method using Milton Roy Spectromic 1201 spectrophotometer with Lipid Peroxide (Malondialdehyde) kit supplied by Bio diagnostic (cat. No. MD2529, Lot no.4801).
b) Ascorbic acid level as antioxidant by high performance liquid chromatography (HPLC) using GBC U.V/ vis detector, GBC LC1110 pump and software: WinChrome Chromatography Ver. 1.3.The separation was done in HPLC Unit / RCMB / Al Azhar University.

Statistical analysis:

The data were collected, revised and presented as mean and standard deviation using the statistical package for social science (SPSS) version 16. The antioxidant and oxidation products serum levels in pre and postoperative blood samples were statistically compared with each other using the paired sample t-test and with those of control group using the independent sample t-test.

3. Results:

The study group was made up of 15 children (9 boys and 6 girls, with a mean age of 3.7 years). The control group consisted of 8 boys and 7 girls with a mean age of 4 years. The differences between sex distributions and mean ages of both groups were statistically insignificant (*P*-value > 0.05). The preoperative serum level of MDA in patients was statistically significantly higher than in controls (*P*-value < 0.05). While there was no statistically significant difference between the post operative serum level of MDA in the patients and its level in the controls (*P*-value = 0.126) (Table 1). Concerning the comparison between the pre operative and post operative serum levels of MDA in the patients group, there was a highly statistically significant difference between them (*P*-value < 0.05) (Table 2). As regards the serum level of ascorbic acid, it was statistically significantly higher in the patients preoperatively than in controls (*P*-value < 0.05), and was statistically significantly lower in them postoperatively than in controls (*P*-value < 0.05) (Table 3). The differences between Preoperative and postoperative serum levels of ascorbic acid in the patients group was highly statistically significant (*P*-value < 0.05) (Table 4).

Table (1): The mean serum levels of MDA in patients and controls.

Groups	MDA		Patients vs controls	
	Mean	±SD	t	*p*-value*
Controls	1.51	±0.52	–	–
Preoperative patients	3.08	±1.37	4.141	0.000
Postoperative patients	1.99	±1.06	1.577	0.126

*** *P*-value ≤ 0.05 is considered significant.**

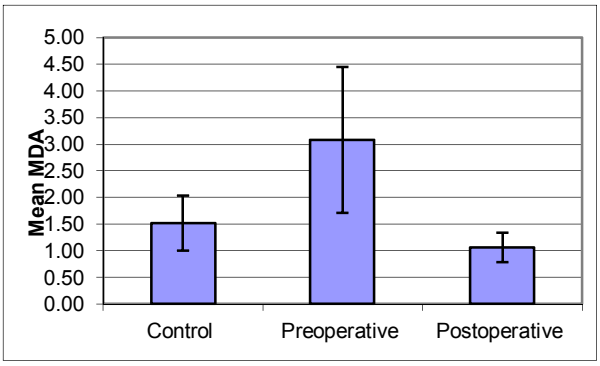

Figure (1): Comparison between control, pre and post operative mean MDA serum levels

Table2: The pre and post operative mean serum levels of MDA in patients

Groups	Mean	±SD	Paired t-test	*p*-value
Preoperative MDA	3.08	±1.37	3.789	0.002
Postoperative MDA	1.99	±1.06		

*** *P*-value ≤ 0.05 is considered significant.**

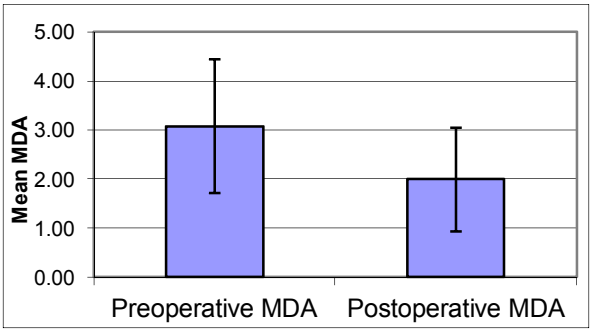

Figure 2: Comparison between the pre and post operative mean MDA serum levels.

Table (3): The mean serum levels of ascorbic acid in patients and controls.

Groups	Ascorbic acid		Patients vs controls	
	Mean	±SD	t	*p*-value
Controls	0.28	±0.11	-	-
Preoperative patients	0.03	±0.02	-8.463	0.000
Postoperative patients	0.09	±0.04	-5.990	0.000

**** P-value ≤ 0.05 is considered significant.***

Table (4): The pre and post operative mean serum levels of ascorbic acid in patients

Groups	Mean	±SD	Paired t-test	*p*-value
Preoperative Ascorbic acid	0.03	±0.02	-7.320	0.000
Preoperative Ascorbic acid	0.09	±0.04		

*** *P*-value ≤ 0.05 is considered significant**

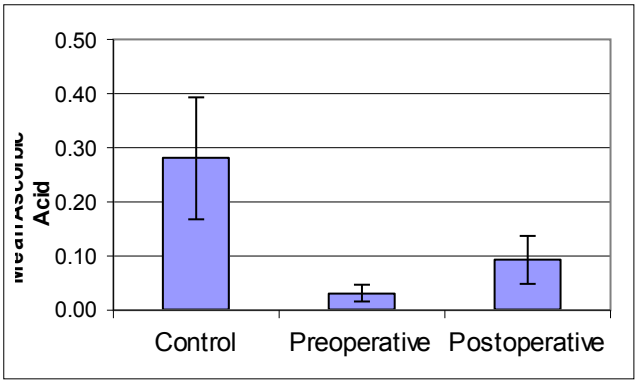

Figure (3): Comparison between control, pre and post operative mean ascorbic acid serum levels

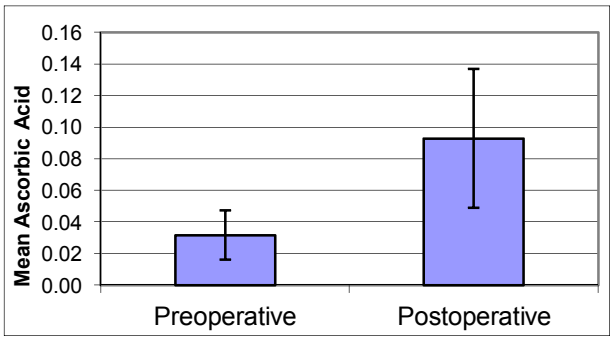

Figure 4: Comparison between the pre and post operative mean ascorbic acid serum levels.

4.Discussion:

Attacks of acute tonsillitis are among the important health problems in preschool and school aged children. The social and physical morbidity they cause is significant. If they do not respond to medical treatment and disturb the life of the patient significantly, the treatment of choice is surgery. Despite the frequency of this disease and the significant morbidity it causes its pathogenesis is not exactly known. [7,8]

Oxidation products are produced within the cell during various cellular reactions. Chemical substances, drugs, radiation, oxygen, cellular aging and phagocytes lead to production of oxidants. The oxidation products are produced during inflammation and are involved in the tissue injury due to this inflammation. [9]

It has been known that lipid peroxidation gives complex products including hydroperoxides, cleavage products such as aldehydes and polymeric materials, and these products that exert cytotoxic and genotoxic effects. [10]

With increasing evidence indicating the involvement of lipid peroxidation in various disorders and diseases, the biomarkers for lipid peroxidation have gained increasing attention. Various markers have been proposed and applied. Isoprostanes (IsoPs), which are

prostaglandin F2-like compounds, and neuroprostanes (NPs) that are formed by the non-enzymatic, free radical-mediated oxidation of arachidonates and docosahexaenoates, respectively, are regarded as the ''gold standard'' for assessing oxidative stress *in vivo*. Similar products that are characterized by a substituted tetrahydrofuran ring structure and termed isofurans (IsoFs) have also been measured and found to increase with increasing oxygen tension, in contrast to IsoPs .[11]

The level of lipid peroxidation products *in vivo* is determined by the balance between their formation, metabolism, secondary reactions and excretion. These biomarkers are also useful for evaluating the beneficial effects of antioxidant foods, spices, beverages, supplements and drugs.[12]

Free radical damage to the membrane lipids of leucocytes leads to increased permeability, and therefore decreases their immune function. DNA damage by free radicals decreases synthesis of certain critical factors by leucocytes and decrease reproductive capacity of leucocytes. [13]

The reduction of lipid hydroperoxides is necessary not only for the prevention of further oxidation and decomposition with concomitant formation of free radicals but also for cell signaling in a regulated and compartmented manner.[14]

Cells have a system for the reduction of lipid hydroperoxides that is composed of diverse antioxidant enzymes with different structures, substrate specificity and localization. Antioxidants within cells, cell membranes and extracellular fluids can be up-regulated and mobilized to neutralize excessive and inappropriate free radical formation.[15]

Antioxidant defenses may be divided into four categories: prevention of the formation of active oxidants, scavenging, quenching and removal of active oxidants, repair of damage and excretion of toxic oxidation products, and adaptive responses. The inhibition of enzymatic lipid oxidation may be achieved by inhibition of either the activation or reaction of an enzyme. Free radical-mediated lipid peroxidation may be inhibited by the inhibition of chain initiation and chain propagation and/or acceleration of chain termination.[16]

Vitamin C, also referred to as ascorbic acid or ascorbate, belongs to the water-soluble class of vitamins. Vitamin C is the major water-soluble antioxidant within the body. It can regenerate other antioxidants and act as an antioxidant itself. [17] Vitamin C readily donates electrons to break the chain reaction of lipid peroxidation. The water-soluble properties of vitamin C allow for the quenching of free radicals before they reach the cellular membrane. Vitamin C has the ability to sequester the singlet oxygen radical and stabilize the hydroxyl radical. [18]

Another important function of vitamin C is to reduce the vitamin E radical to regenerate vitamin E and also to inhibit the prooxidant action of vitamin E. This interaction proceeds efficiently for the vitamin E radical in the membrane and low density lipoprotein (LDL). [19]

Tocopherol and glutathione also rely on vitamin C for regeneration back to their active isoforms. The relationship between vitamin C and glutathione is unique. Vitamin C reduces glutathione back to the active form. Once reduced, glutathione will regenerate vitamin C from its oxidized state. [20]

Cells exhibit a broad spectrum of responses to oxidative stress, depending on the stress type and level encountered. Oxidative stress exceeding the antioxidant capacity level may induce oxidative damage, but low-level stress may enhance the defense capacity. Such an adaptive response has been observed in several instances, particularly in low-dose irradiation. [21]

Many investigators reported that during chronic inflammatory processes antioxidants decrease slowly when the level of oxidative stress they can neutralize is exceeded. Since CT is a chronic inflammatory disease, there is a significant possibility that the balance between oxidation products and antioxidants is involved in the appearance and chronicity of this disease. [13]

This study demonstrated a statistically significant high serum level of lipid peroxidation product (MDA) as a marker of oxidation stress and a statistically significant low serum level of antioxidant (ascorbic acid) in children with CT before tonsillectomy compared with control group. This high level of MDA may be due to chronic inflammatory state. In agreement with our results Yilmaz *et al.* [13] reported decreased preoperative blood level of vitamin C and increased preoperative blood level of MDA.

Our results showed that the decreased preoperative serum level of ascorbic acid significantly increased postoperatively, and that increased preoperative serum level of oxidation product MDA significantly decreased post operatively. However, these levels never reached those of the control group. This was parallel to the findings of Yilmaz *et al.* [13], who concluded that antioxidant capacity of the study group increased but not normalized 1month after the operation and that the oxidative stress decreased but still continued. Probably 1 month was not long enough for the oxidative stress or antioxidant capacity to normalize. Nevertheless, significant postoperative improvement

in the oxidative stress indicated that tonsillectomy removed microbial sources in these children and thus, decreased general oxidative stress. This operation also might have supported the immune system of these children.

Shukla *et al.* [5] determined that in adolescents with chronic tonsillitis, the post operative blood level of MDA decreased.

Also Aleszczyk *et al.* [22] found that in patients with CT who developed complications or who had severe attacks were shown to have lower blood levels of vitamins B and C than healthy people. These patients also had decreased activity of cellular immune system and leukocyte chemotaxis.

In conclusion: low blood level of antioxidant and high blood level of oxidation product in patients with CT, and the tendency of blood levels to normalize after surgery indicate that they are involved in the pathogenesis of CT .

Finally we recommend:

1. Further studies to evaluate their possible therapeutic role in preventing recurrent tonsillitis.
2. Giving ascorbic acid post tonsillectomy to help normalize their blood level.

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