

A Comparison of Lucigenin - Free Oxygen Radicals in Reactive Hyperplasia and Recurrent Tonsillitis

Reaktif Hiperplazi ve Rekürrent Tonsillitlerde Lusigenin - Serbest Oksijen Radikallerinin Karşılaştırılması

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ABSTRACT

Aim: Today, tonsillectomy (T) used for chronic tonsillitis is among the most common surgery type that ear, nose, and throat physicians perform. The differences in the pathogenesis of chronic tonsillitis and tonsil hypertrophy are still unknown. This study aimed to compare the levels of free oxygen radicals in patients with tonsil hypertrophy and chronic tonsillitis and to investigate the role of free oxygen radicals in the pathogenesis of chronic tonsillitis.

Materials and Method: The study included 40 patients who had chronic tonsillitis indications based on Paradise criteria, and T was performed. Operation materials were histopathologically examined, and their free oxygen radicals (FOR) levels were measured.

Results: Based on the histopathological results, the participants were divided into two groups: one including 28 (70%) patients who had active or chronic tonsillitis, the other including 12 (30%) patients with reactive follicular hyperplasia. Lucigenin - free oxygen radicals (Luc-FOR) levels of the group with chronic tonsillitis and that of the group with reactive follicular hyperplasia were 82.29 ± 32.3 Auc and 50.76 ± 7.95 Auc, respectively. Luc-FOR values were found to be significantly higher in the group with chronic tonsillitis ($p=0.001$).

Conclusion: Free oxygen radicals play an essential role in tonsillar pathologies. Differences in the Luc-FOR levels measured from tonsillar tissue in patients with tonsil hypertrophy and chronic tonsillitis demonstrate that the pathogenesis of these two diseases is different.

ÖZET

Giriş ve Amaç: kronik tonsilite bağlı Tonsillektomi (T), Kulak Burun Boğaz hekimlerinin en sık yaptıkları ameliyatlardandır. Halen kronik tonsillit ve tonsil hipertrofisi patogenizindeki farklılıklar tam olarak bilinmemektedir. Tonsil hipertrofisi ve kronik tonsillit hastalarında serbest oksijen radikallerinin seviyelerinin karşılaştırılması ve serbest oksijen radikallerinin kronik tonsillit patogenezindeki rolünün araştırılması bu çalışmada amaçlanmıştır.

Gereç ve yöntem: Çalışmaya Paradise kriterlerine göre Tonsillektomi (T) endikasyonu konulan ve opere edilen 40 hasta alındı. Operasyon materyallerine histopatolojik inceleme ve serbest oksijen radikallerden süperoksit ölçümleri yapıldı.

Bulgular: Histopatolojik bulgulara göre; 28 (%70) hastadan oluşan aktif yada kronik tonsillitli diğeri ise 12 (%30) hastadan oluşan reaktif folliküler hiperplazili olarak iki gruba ayrıldı. Kronik tonsillitli grubun lusigenin-serbest oksijen radikal (Luc-SOR) değeri 82.29 ± 32.3 Auc, reaktif folliküler hiperplazi'li grubun ise 50.76 ± 7.95 Auc bulundu. Luc-SOR değeri karşılaştırıldığında kronik tonsillitli grupta anlamlı derecede ($p=0.001$) yüksek olduğu belirlendi.

Sonuç: Serbest oksijen radikalleri tonsiller patolojilerde önemli rol oynamaktadır. Tonsil hipertrofisi ve kronik tonsillit hastalarının tonsil dokusundan ölçülen Luc-SOR değerlerinin farklı olması bu iki hastalığın patogenezinin de farklı olduğunu göstermektedir.

Key Words:

Tonsillectomy,
Chronic tonsillitis,
Free oxygen radicals.

Anahtar Kelimeler:

Tonsillektomi,
Kronik tonsilit,
Serbest oksijen radikalleri.

INTRODUCTION

Palatine tonsils, a part of the Waldeyer ring, are the most common cause of upper respiratory tract infections (1). Beta-hemolytic streptococci, Streptococcus pneumoniae, and Haemophilus influenzae are the most common bacterial agents that cause acute tonsillitis (2, 3). Characteristic symptoms of acute tonsillitis are sudden onset of fever, painful cervical lymphadenopathy with sore throat and erythematous tonsils including yellow-white purulent exudate in the

crypts. Symptoms of chronic tonsillitis include having acute tonsillitis attacks for 4 or 5 times a year, feeling of continuous throat pain, difficulty in swallowing, weakness, atrophy and/or hypertrophy in tonsils and tonsil magma (4). The host first responds with polymorphonuclear leukocytes (PMNL) and antibodies during the chronic process. Then, as a result of the complex interaction between pathogenic bacteria and the host immune response, cytokines, prostanooids, and proteinases, causing local and systemic tissue damage,

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are secreted by PMNLs (5). PMNLs produce free oxygen radicals (FOR) during the bacterial phagocytosis. Neutrophils show their antimicrobial effects in two different mechanisms. These are oxygen-dependent and oxygen-independent mechanisms. The generation of FOR with oxygen-dependent mechanism begins with activation of Nicotinamide Adenine Dinucleotide Phosphate-H (NADPH) oxidase enzyme in the plasma membrane of PMNLs (6). PMNLs are produced by stimulation of the bacterial antigen, and superoxide and hydrogen peroxide radicals are produced by catalyzing the NADPH enzyme during phagocytosis (7). Oxygen-independent mechanism does not include oxygen molecule consumption and obsolete oxygen metabolites (8). FOR can occur both in regular metabolic events in organisms and during phagocytosis, especially in chronic infections (9).

Tonsillary pathologies have two different diseases: chronic tonsillitis and tonsillar hypertrophy. Although these two diseases originate from the same tissue, they show different clinical and different histopathological findings (10). Furthermore, the pathogenesis of these two diseases and the role of free oxygen radicals in this pathogenesis have not been elucidated. This study aimed to compare the Lucigenin - free oxygen radicals (Luc-FOR) levels in tonsillectomy specimens by using the chemiluminescence (KL) method in patients with tonsil hypertrophy and chronic tonsillitis and to investigate the role of free oxygen radicals (FOR) in the pathogenesis of chronic tonsillitis.

MATERIAL AND METHODS

The study included 40 patients who were admitted to our clinic with the complaint of tonsillitis between 2010 and 2018. The participants met Paradise criteria, and they did not smoke (11). All patients were performed T under general anesthesia. During the period when the operation was performed, all the patients were protected against acute tonsillitis and/or respiratory tract infection. For histopathological examination, the excised materials were placed in containers containing 10% formaldehyde, and they were placed in special tubes for FOR analysis. FOR measurement was performed using the KL method. KL is a noninvasive and direct method for the determination of FOR in tissues and cells. KL measurements are carried out using devices called luminometers. Luminometers contain sensitive light detectors. When PMNLs meet bacteria, oxygen consumption increases, and FOR molecules and light energy emerge. Luminometers measure this light energy (12, 13). The samples were placed in count tubes containing 0.5 M phosphate-buffered saline (PBS) and 20 mM HEPES. Superoxide radicals were measured using lucigenin (Luc) (Acros Lab., France) in a luminometer (EG and Berthold Junior LB 9509). Counting tubes were placed in the luminometer in one-minute intervals for five minutes, their dry weights were measured, and the area under curve (Auc) rlu/mg

tissue was calculated by calculating the area under the curve. In the calculation of this area, the Auc value is determined by taking the integral of the measurement points using the following formula.

$$\text{Area} = \Delta t[(y_1/2 + y_2 + y_3 + \dots + y_{n-1} + (y_n/2))]$$

Δt : time interval

n: total number of measurement points

y_1, y_2, \dots, y_n : measurements

Homogeneity of the groups was evaluated using the 'Mann-Whitney U-test,' and the correlations between them were analyzed using the Pearson test. The significance of the results was evaluated at $p < 0.05$.

The study was approved by the Istanbul Yeni Yüzyıl University Ethics Board (Date: January 07, 2019 - Decision No: 2019/01).

RESULTS

In our study, 25 (62%) patients among 40 whom T was performed were male, 15 (38%) were female, and their age was between 15 and 43 years (18.6).

Histopathological examination revealed that 28 (70%) patients had active or chronic tonsillitis (figure 1), and 12 (30%) had reactive follicular hyperplasia (figure 2).

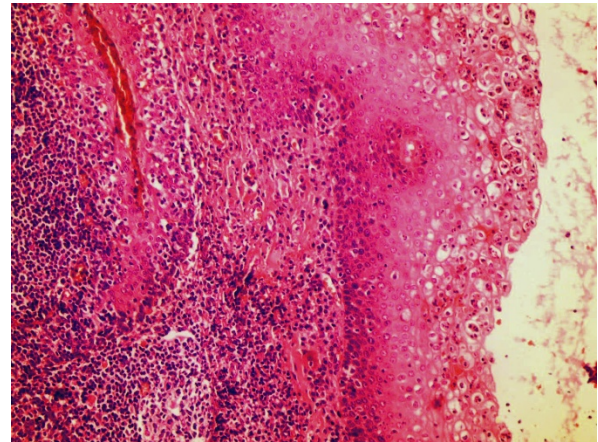


Figure 1: Acute and or chronic tonsillitis (Hematoxylin and eosin staining X40)

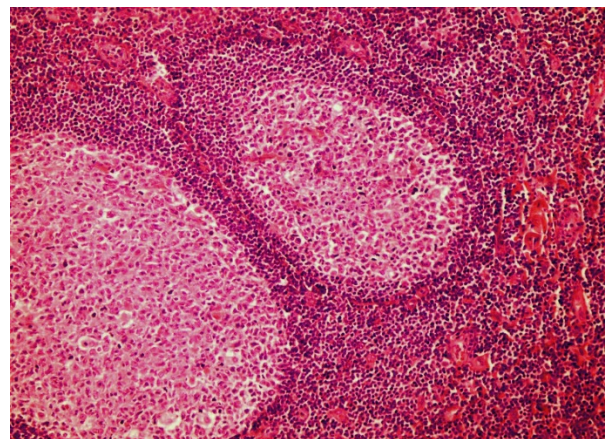


Figure 2: Lymphoid hyperplasia (Hematoxylin and eosin staining X40)

The patients were divided into two groups based on the histopathological findings, and their Luc-FOR values were examined.

Luc-FOR value of the group with chronic tonsillitis group was 82.29 ± 32.3 Auc, and that of the group with reactive follicular hyperplasia was 50.76 ± 7.95 Auc. It was found that Luc-FOR values were significantly higher in the group with chronic tonsillitis ($p=0.001$) (Table 1).

DISCUSSION

Tonsillectomy is still among the most frequently performed operations during childhood in the US and Europe (14, 15). In our clinic, 15.4% of the operations performed between 2010 and 2018 was tonsil operation.

Indications for tonsil operations were classified as i) chronic and/or recurrent tonsillitis, ii) upper respiratory tract obstruction, iii) suspicion of malignancy, iv) other rare causes. Based on the Paradise criteria, the first group consisted of the patients who had acute tonsillitis with fever 7 or more times in the last year, those who had acute tonsillitis with fever 5 or more times in two consecutive years, and those who had acute tonsillitis with fever 3 or more times in following 3 years. Also, all the patients' used antibiotics, and the illness lasted one week (11, 16). In our study, we selected patients with chronic tonsillitis based on these criteria.

PMNLs produce a significant amount of FOR in the process of chronic tonsillitis during the bacterial phagocytosis. Molecular oxygen is the primary source of FOR. As a result of the reduction of oxygen, reactive oxygen derivatives such as superoxide, hydrogen peroxide, and hydroxyl radicals emerge. These cytotoxic structures cause oxidative damage in cells. Biological effects of the reactive oxygen species are controlled by enzymatic defense mechanisms such as superoxide dismutase (SOD) (17). The superoxide anion is the most important FOR released from the activated inflamed cells (18, 19). Since it cannot penetrate lipid membranes, it does not engage in the systemic circulation and remains where it is produced (9, 20-22). In our study, we showed that the fact that superoxide radical does not engage in the systemic circulation and that it is an important FOR marker in the pathogenesis of chronic tonsillitis, and histopathological findings support this.

Unstable FOR types attack the cells to stabilize and result in various diseases, damaging cell components. The superoxide radical, which has no harmful effect,

shows this quality as the source of hydrogen peroxide and the reductor of transition metal ions (23). FOR also react with lipids, proteins, carbohydrates, and DNA to get electron. When the number of these oxidants is too high, they cause peroxidation of the lipids in the membrane leading to disruption of the permeability and, thus, to intracellular ion imbalance. They disrupt the structure of DNA and RNA molecules and play an essential role in the formation of many cancer cells (24).

In our study, we conducted the histopathological examination and measurement of superoxide radical measurements of the operation material of 40 patients who were diagnosed with chronic and/or recurrent tonsillitis and whom T was applied based on the Paradise criteria. Among the patients, 28 (70%) had chronic tonsillitis, and 12 (30%) had reactive follicular hyperplasia. The levels of superoxide radicals were significantly higher in the group with chronic tonsillitis.

In the literature, although there are much researches (18, 25-33) investigating the high levels of FOR in tonsillectomy materials, there is only one article conducted by Kiroglu et al. (34) examining the difference of oxidant levels between chronic tonsillitis and tonsil hypertrophy. In this study, under our article, tonsil oxidase (malondialdehyde) and anti oxidase (catalase) levels were found to be significantly high in patients with tonsillitis compared to those with hypertrophy. However, in the same study, no significant difference was found in serum oxidase levels between patients with chronic tonsillitis and tonsillar hypertrophy. This finding shows that the superoxide radical activity is in tonsillar tissue rather than blood. In a study conducted by Cvetkovic et al. (33) investigating the oxidative stress in patients with tonsillar hypertrophy and chronic tonsillitis, lipid peroxidation was found to be higher in the blood levels compared to the control group, moreover no significant difference in lipid peroxidation levels was found between patients with tonsillar hypertrophy and chronic tonsillitis. However, in this study, because only blood levels were examined, the increase in serum levels of oxygen radicals in cases with tonsillar hypertrophy may be due to the stress of the body after exposure to airway obstruction. In other studies in the literature, the relationship between preoperative and postoperative blood levels of oxidants and antioxidants was investigated. In the researches conducted by Shukla et al.(18), Kaygusuz et al. (32) and Yilmaz et al. (31) it was revealed that pre-operative levels of

Table 1: FOR values

	The group with active or chronic tonsillitis	The group with reactive follicular hyperplasia	P
Luc.FOR	86.29 ± 32.3 Auc	50.76 ± 7.95 Auc	0.001

Luc.: lucigenin FOR: free oxygen radicals Auc: area under the curve

malondialdehyde increased significantly compared to post-operative because of oxidative stress due to inflammation after tonsillectomy and it was shown that serum SOD levels decreased after surgery. In the study conducted by Yilmaz et al. (31), FOR levels decreased after T but did not reach the level of healthy patients in the long term. In this study, it is recommended that perioperative antioxidant treatment should be discussed for the faster recovery of patients. However, in a similar survey by Dogruer et al. (30), although serum malondialdehyde levels decreased after T compared to pre-operative levels, SOD levels did not change significantly. This was attributed to defense mechanisms on antioxidant levels as a result of long-term exposure to hypoxia. According to Shukla et al. (35), antioxidant therapy is as effective as

tonsillectomy, and the application of perioperative antioxidant treatment should be discussed.

In conclusion, few studies are investigating the role of FOR in the pathogenesis of tonsillar hypertrophy and chronic tonsillitis. In our research, it was shown in parallel with previous studies that tonsillar FOR levels were higher in patients with chronic tonsillitis compared to patients with tonsillar hypertrophy and the pathogenesis of tonsillar hypertrophy and chronic tonsillitis were different even if they originated from the same tissue. The role of the tonsil tissue FOR levels in determining the correct tonsillectomy indication should be discussed in further researches.

Conflicts of Interest

All other co-authors have no conflicts of interest

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