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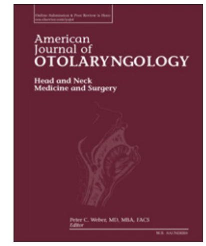

Evaluation of iron and zinc levels in recurrent tonsillitis and tonsillar hypertrophy

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ABSTRACT

Objectives: The aim of this study is to look into the roles of iron and zinc metals in etiopathogenesis of recurrent tonsillitis and tonsillar hypertrophy by evaluating the levels of iron and zinc elements in the palatine tonsillar tissue.
Methods: In total, 40 patients who underwent a tonsillectomy to treat recurrent tonsillitis and tonsillar hypertrophy were included in the study. Patients were classified into two groups, recurrent tonsillitis and tonsillar hypertrophy, determined by the results of clinical and histopathological examination. The levels of iron and zinc elements were determined for each tonsillar tissue sample.
Results: There was a significant difference in the iron and zinc concentrations ($p < 0.001$) between the tonsillar hypertrophy and recurrent tonsillitis groups. The levels of iron and zinc were significantly lower in the recurrent tonsillitis group.
Conclusions: This study suggests that low tissue concentrations of iron and zinc may lead to recurrent tonsillitis.

1. Introduction

Palatine tonsils are the most important constituent of the specialized lymphoid organs located at the upper respiratory tract, which are called the Waldeyer's ring. Infectious and hypertrophic diseases of the palatine tonsils are the pathologies most frequently encountered by ear, nose and throat specialists. As there are authors advocating that tonsillar hypertrophy (TH) and recurrent tonsillitis (RT) are histopathologically different, there are also others subscribing to the opposite opinion [1,2]. As TH and RT are clinically two different entities; in addition to histopathological differences, they were investigated in terms of oxidants–antioxidants, apoptosis and gene polymorphism in different studies [3–6].

Iron is an essential element for the development of the immune system. Iron is the most important component of peroxidase and the enzymes producing nitrous oxide, which are vital for the enzymatic function of immune system cells. Moreover, iron plays a critical role in the regulation of cytokine production and in the development of cellular

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immunity [7]. Zinc has three important biological functions: catalyzer, structural and regulatory; it is one of the most valuable trace elements for the organism. Zinc homeostasis has critical effects on immune function, oxidative stress and apoptosis. Zinc is also essential for the activities of many enzymes including superoxide dismutase, carbonic anhydrase, and matrix metalloproteinases. The levels of iron and zinc in the body have been shown to influence various diseases including malignancies, degenerative diseases and infectious diseases [7–11].

There are a limited number of studies investigating trace elements in tonsillar tissue [12,13]. The aim of this study is to evaluate the levels of iron and zinc elements in the palatine tonsillary tissue and to look into the roles of iron and zinc metals in the etiopathogenesis of RT and TH.

2. Materials and method

2.1. *Subjects*

Forty tonsillar tissue samples obtained from 40 patients, who were operated on for tonsillar disease in the Gaziosmanpaşa University Faculty of Medicine ENT Department were included in the study. The hospital ethics committee approved the study and written informed consent was obtained from the patients after the nature and purpose of the study had been fully explained to them. All experiments were performed according to the Declaration of Helsinki. Tonsillectomy operations were performed using the cold dissection method under general anesthesia on every patient. Following the operation, a tissue sample that contained both core and superficial regions of palatine tonsil was obtained, and stored at −20 °C until the time of analysis. According to their diagnoses, 20 patients were categorized as RT and 20 patients were categorized as TH. Clinical and histopathological parameters were used together for the diagnosis of RT and TH. Patients in whom clinical parameters did not show correlation with histopathological findings were excluded from the study.

2.2. *Clinical evaluation*

Tonsillar size was classified according to the Friedman staging system as stage 1, 2, 3 and 4 [14]. In addition to having stage 3 or 4 tonsillar size, patients who had symptoms of open-mouth breathing during sleep, snoring, and symptoms of obstructive sleep apnea were accepted as TH. In addition to having Friedman stage 1 or 2 tonsillar size, patients with RT had well-documented, clinically confirmed and appropriately treated acute tonsillitis episodes — at least seven episodes in the past year or at least five episodes per year for two years, or at least three episodes per year for three years [15].

2.3. *Histopathological examination*

Tonsillar tissues were transferred to the pathology laboratory in a 10% formalin solution. After routine specimen tracking procedures, tissues were embedded in paraffin. Sections at 4 μm thickness were obtained, and were stained with hematoxlin–eosin and examined under a light microscope.

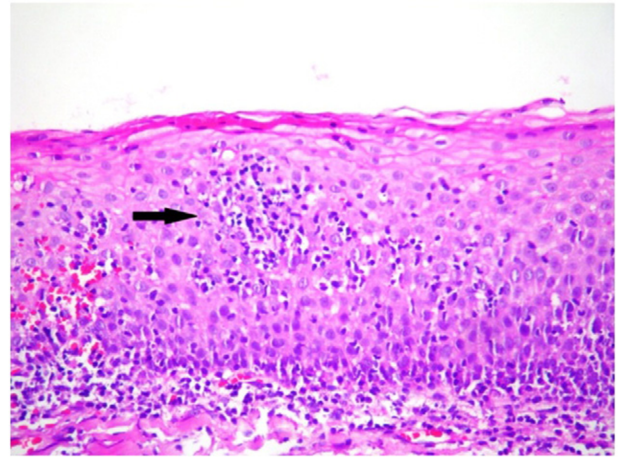


Fig. 1 – Increased number of lymphoid follicular structures in subepithelial stroma.

In the tonsillar tissues, surface epithelia, crypt epithelia and stroma were evaluated histopathologically, and presence of lymphocytes, polymorphonuclear leukocytes, plasma cell infiltration and fibrosis was determined in these areas. According to this evaluation, cases with significant lymphocytic infiltration at the surface epithelium and defects at the surface and crypt epithelia were diagnosed with RT (Fig. 1), whereas those with a prominent increase in lymphoid follicles in the stroma were accepted as TH (Fig. 2).

2.4. *Analytical procedure*

All plastic and glass equipment used in the study were kept in 10% nitric acid solution for 18 h and were properly rinsed out with deionized water. A 1 g sample was obtained from the tonsillar tissue of each specimen. This sample was digested with 3 mL of HNO3 (Suprapure) and 1 mL of H2O2 (Suprapure) in a Milestone Ethos D closed-vessel microwave digestion system for 31 min (the digestion conditions used in the microwave system were 250 W for 2 min, 0 W for 2 min, 250 W for 6 min, 400 W for 5 min, 550 W for 8 min, vent for

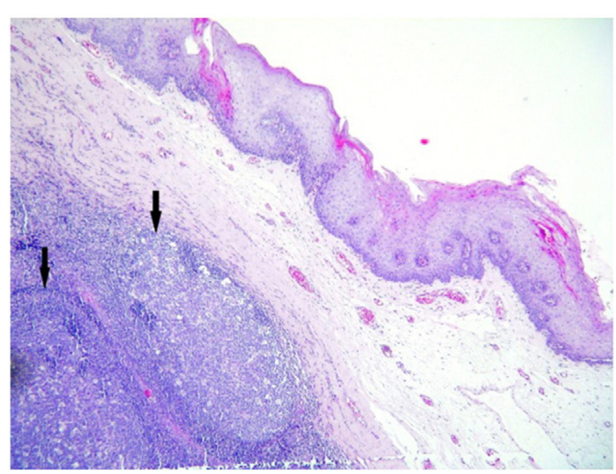


Fig. 2 – Prominent lymphocytic infiltrate at surface epithelium.

8 min) and diluted to 5 mL with double-deionized water (Milli-Q). A blank digest was carried out in the same way. This procedure was used because of its high accuracy in both time and recovery. The recovery values were nearly quantitative (C95%) for this digestion method. The relative standard deviations were less than 10% for all elements. A Perkin Elmer Analyst 700 atomic absorption spectrometer (FAAS; Perkin-Elmer, Waltham, MA) equipped with an HGA graphite furnace and a deuterium background corrector was used. The iron and zinc levels in the samples were determined by the graphite furnace AAS. For the graphite furnace measurements, argon was used as an inert gas. Pyrolytic-coated graphite tubes (Perkin Elmer part number B3001264) were used with a platform, and signals were measured at the peaks. Results were calculated as μg/g wet tissue. All analyses were performed by the same examiners who were unaware of the nature of the samples. All samples were assayed in duplicate.

2.5. *Statistical analysis*

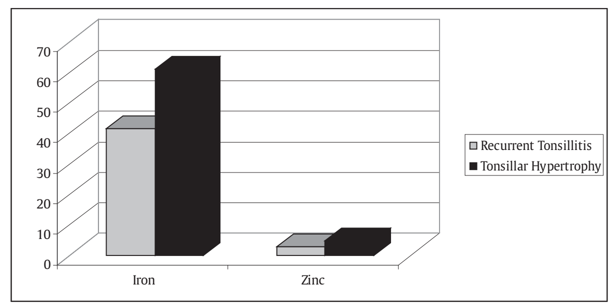
The Statistical Package for Social Sciences software (SPSS, version 20.0 for Windows; SPSS Inc., Chicago, IL) was used to perform all analyses. The descriptive statistics used were mean and standard deviations. Comparisons of values between patients were performed using the Mann–Whitney U test. Probability values of less than 0.05 were considered significant.

Table 1 – Distribution and comparison of iron and zinc concentrations in recurrent tonsillitis and tonsillar hypertrophy group.

a

Group	Recurrent Tonsillitis Mean (min-max)	Tonsillar Hypertrophy Mean (min -max)
İron (μg/g)	41.46 (28.60-87.87)	61.19 (28.69-113.40)
Zinc (μg/g)	2.77 (1.118-3.90)	4.91 (2.13-7.81)
p value	< 0.001	< 0.001

b



3. Results

The tissue samples of 40 patients (20 RT, 20 TH) were included in the study. There were 20 patients (16 male, 4 female) in RT group, and the mean age was 10 ± 3 years. There were 20 patients (10 male, 10 female) in TH group and the mean age was 13 ± 3.5 years. There was no significant difference with regards to age (p = 0.620) and sex (p = 0.183) in either group. Trace metal concentrations were determined as a wet weight and measured in micrograms per gram.

There were significant differences in iron and zinc concentrations between the TH and RT groups (p < 0.001). Iron and zinc levels were significantly low in the RT group. The metal concentrations in the TH samples were 61.19 ± 4.99 and 4.91 ± 0.34 μg/g for iron and zinc, respectively. The metal concentrations in the RT samples were 41.46 ± 2.94 and 2.77 ± 0.18 μg/g for iron and zinc, respectively. When iron concentrations were compared to zinc concentrations in both TH and RT samples, the iron concentrations were higher. Tissue levels of iron and zinc for all patients are compared in Table 1a and b.

4. Discussion

In Ear, Nose and Throat practice, one the most important pathologies of the Waldeyer's ring in both pediatric and adult age groups is the diseases of the palatine tonsil. Tonsillectomy has been an important treatment option for these diseases for so long [15,16]. TH and RT are the two most frequent indications for tonsillectomy [4]. Although the pathogenesis of TH and RT has not been clearly understood, it has been noted that these two diseases are two different entities [1]. After this point, TH and RT have been compared to each other in various studies. Kiroğlu et al. compared these two diseases for oxidants and anti-oxidants, and found that malondialdehyde and catalase levels were higher in the infectious group [4]. Gürbüzler et al. [3] compared TH and RT for manganese-superoxide dismutase and glutathione peroxidase 1 polymorphism; another study examined apoptosis [6]. In the aforementioned studies, generally clinical findings were taken into account for the differentiation of RT and TH, while pathological findings were overlooked. In our study, however, clinical findings were correlated to pathological findings for the differentiation of TH and RT. In the histopathological evaluation of tonsillary tissue, those cases with significant lymphocytic infiltrate in the surface epithelium and with defects in surface and crypt epithelia were accepted as RT, while cases with a prominently increased number of lymphoid follicles in stroma were accepted as TH. Kutluhan et al. [1] emphasized the same findings for the differentiation of TH and RT in their study. They determined that crypt epithelium defects were more frequently compared to the other findings.

Some studies have shown that TH and RT result from the hypofunction of local and systemic immunity [17,18]. Iron is an important element for our immune system: it plays a role in both cellular and humoral immunity. Iron is essential for normal proliferation and the growth of the tissues which are a physical barrier against infections and have high turnover. Since iron is also an essential requirement for most human pathogens, there are host defense mechanisms that withhold existing iron from the pathogens [19–22]. Iron is also the most important component of the enzymes producing peroxidase and nitrous oxide, which are pivotal for our immune system [6,7,23]. One study showed that iron deficiency posed a risk for respiratory diseases, otitis media and acute adenoiditis in infants [23,24]. In our study, iron levels were significantly lower in the RT group compared to the TH group. Low iron concentrations in tonsillary tissue of RT group might have created a tendency toward frequent infections. Additionally, this might also have resulted from utilization of iron by the microorganisms due to frequent infections.

Zinc homeostasis has critical effects on immune function, oxidative stress and apoptosis. Zinc regulates leukocyte function and cytokine expression, and plays an essential role in immune function. Zinc is also essential for the activities of many anti-oxidant enzymes such as superoxide dismutase, carbonic anhydrase and matrix metalloproteinases [8]. Some studies have shown an increased risk for infectious diseases in cases where zinc deficiency is present [25]. Önerci et al. [12] compared the levels of trace elements between patients with RT and chronic tonsillitis and a control group, and found that zinc levels were significantly lower in the patient group than in the control group. They noted the correlation between serum and tissue concentrations of zinc in their study. We found the zinc concentration in tonsillary tissue samples to be significantly lower in the RT group compared to the TH group. This result indicates that as zinc is an element essential to the immune system, lower levels of zinc could predispose the organism to infections.

There are two limitations to our study. First, the serum levels of the elements were not evaluated in these patients. However, it was intended in this study to examine the difference between the TH and RT groups of the element concentrations at tissue level. Secondly, we could not compare the tissue element concentrations measured in the TH and RH groups with the concentrations in normal tonsillar tissue.

In conclusion, the present study is first study comparing the tissue element concentrations in RT and TH correlated with histopathology and clinically. This study showed that low tissue concentrations of zinc and iron could create a predisposition to recurrent tonsillitis due to their basic contributions to immune system. We believe that studies comparing the concentrations of iron, zinc and other elements in normal tonsillar tissues obtained from cadavers to those obtained from RT and TH groups would further contribute to our knowledge base.

REFERENCES

[1] Kutluhan A, Ugras S, Kiris M, et al. Differences in clinical and histopathologic features between chronic adenotonsillitis and chronic adenotonsillar hypertrophy. Kulak Burun Bogaz Ihtis Derg 2003;10:61–7.

[2] Bieluch VM, Martin ET, Chasin WD, et al. Recurrent tonsillitis: histologic and bacteriologic evaluation. Ann Otol Rhinol Laryngol 1989;98:332–5.

[3] Gurbuzler L, Sogut E, Koc S, et al. Manganese-superoxide dismutase and glutathione peroxidase 1 polymorphisms in recurrent tonsillitis and tonsillar hypertrophy. Int J Pediatr Otorhinolaryngol 2012;76:1270–3.

[4] Kiroglu AF, Noyan T, Oger M, et al. Oxidants and antioxidants in tonsillar and adenoidal tissue in chronic adenotonsillitis and adenotonsillar hypertrophy in children. Int J Pediatr Otorhinolaryngol 2006;70:35–8.

[5] Acioglu E, Yigit O, Alkan Z, et al. The role of matrix metalloproteinases in recurrent tonsillitis. Int J Pediatr Otorhinolaryngol 2010;74:535–9.

[6] Onal M, Yilmaz T, Bilgic E, et al. Apoptosis in chronic tonsillitis and tonsillar hypertrophy. Int J Pediatr Otorhinolaryngol 2015;79:191–5.

[7] Kumar V, Choudhry VP. Iron deficiency and infection. Indian J Pediatr 2010;77:789–93.

[8] Namuslu M, Balci M, Coskun M, et al. Investigation of trace elements in pterygium tissue. Curr Eye Res 2013;38:526–30.

[9] Chasapis CT, Loutsidou AC, Spiliopoulou CA, et al. Zinc and human health: an update. Arch Toxicol 2012;86:521–34.

[10] Bray TM, Bettger WJ. The physiological role of zinc as an antioxidant. Free Radic Biol Med 1990;8:281–91.

[11] Bansal A, Parmar VR, Basu S, et al. Zinc supplementation in severe acute lower respiratory tract infection in children: a triple-blind randomized placebo controlled trial. Indian J Pediatr 2011;78:33–7.

[12] Onerci M, Kus S, Ogretmenoglu O. Trace elements in children with chronic and recurrent tonsillitis. Int J Pediatr Otorhinolaryngol 1997;41:47–51.

[13] Torjussen W, Andersen I, Zachariasen H. Nickel content of human palatine tonsils: analysis of small tissue samples by flameless atomic absorption spectrophotometry. Clin Chem 1977;23:1018–22.

[14] Friedman M, Tanyeri H, La Rosa M, et al. Clinical predictors of obstructive sleep apnea. Laryngoscope 1999;109:1901–7.

[15] Baugh RF, Archer SM, Mitchell RB, et al. Clinical practice guideline: tonsillectomy in children. Otolaryngol Head Neck Surg 2011;144:1–30.

[16] Balfour DC. Tonsillectomy in children from the stand-point of the general surgeon. Ann Surg 1915;61:257–60.

[17] Bernstein JM, Rich GA, Odziemiec C, et al. Are thymus-derived lymphocytes (T cells) defective in the nasopharyngeal and palatine tonsils of children? Otolaryngol Head Neck Surg 1993;109:693–700.

[18] Hata M, Asakura K, Saito H, et al. Profile of immunoglobulin production in adenoid and tonsil lymphocytes. Acta Otolaryngol Suppl 1996;523:84–6.

[19] Wander K, Shell-Duncan B, McDade TW. Evaluation of iron deficiency as a nutritional adaptation to infectious disease: an evolutionary medicine perspective. Am J Hum Biol 2009;21:172–9.

[20] Al-Younes HM, Rudel T, Brinkmann V, et al. Low iron availability modulates the course of *Chlamydia pneumoniae* infection. Cell Microbiol 2001;3:427–37.

[21] Nemeth E, Ganz T. Regulation of iron metabolism by hepcidin. Annu Rev Nutr 2006;26:323–42.

[22] Marx JJ. Iron and infection: competition between host and microbes for a precious element. Best Pract Res Clin Haematol 2002;15:411–26.

[23] Tansarli GS, Karageorgopoulos DE, Kapaskelis A, et al. Iron deficiency and susceptibility to infections: evaluation of the clinical evidence. Eur J Clin Microbiol Infect Dis 2013;32:1253–8.

[24] Levy A, Fraser D, Rosen SD, et al. Anemia as a risk factor for infectious diseases in infants and toddlers: results from a prospective study. Eur J Epidemiol 2005;20:277–84.

[25] Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr 1998;68:447–63.