Relationship of endoplasmic reticulum stress with the etiopathogenesis of chronic tonsillitis and tonsillar hypertrophy in pediatric patients: a prospective, parallel-group study

Merih Onal1 · Nadir Kocak2 · Fahrettin Duymus2 · Mete Kaan Bozkurt1 · Cagdas Elsurer1 · Omer Erdur1 · Ozkan Onal3,4

Abstract

Objectives Tonsil tissue is a very important component of the human immunity system, contributing to the functioning of the cellular and humoral defence system, especially in childhood. The endoplasmic reticulum (ER) is an organelle that has a very important function in the balanced functioning of cells, in which the accumulation of a cellular protein called ER stress occurs in case of dysfunction. ER stress influences the pathogenesis of many diseases and immune system functions. We aimed to investigate the relation between the diseases of tonsil tissue and ER stress response to elucidate the mechanisms of diseases related with the immune system.

Methods A prospective study was conducted in 46 children aged between 2 and 16 years who underwent tonsillectomy for chronic tonsillitis or tonsillar hypertrophy. Tonsil tissue was separated into two groups according to their size and evaluated in terms of ER stress markers and apoptosis markers by Real-time PCR and Western blot analysis.

Results The ΔCT levels of ER stress markers (ATF4, ATF6, CHOP, GRP78, EIF2AK3, ERN1, GRP94) were greater in children with chronic tonsillitis (p < 0.005). In contrast, the tonsillar hypertrophy group had greater ΔCT levels of apoptosis markers (BAX, BCL-2) according to the Real-time PCR method (p < 0.005). According to the Western blot analysis, the normalized levels of ATF4, ATF6, CHOP, GRP78, and ERN1 genes were found greater in the chronic tonsillitis group than the tonsillar hypertrophy group. There was no difference between the two groups in terms of normalized BCL-2 and BAX levels by Western blot analysis.

Conclusion This is the first study in the literature investigating the effect of the ER stress pathway on the etiopathogenesis of tonsil diseases. It was concluded that the ER stress pathway plays a role in the etiopathogenesis of chronic tonsillitis. Investigating the relationship between ER stress and structures such as the tonsil tissue that make up the immune system can help create new treatment strategies.

Clinical Trial Registration Trial Registration ClinicalTrials.gov Identifier: NCT04653376.

Keywords Endoplasmic reticulum stress · Apoptosis · Children · Genetics · Palatine tonsil · Tonsillitis

Introduction

Tonsil tissue, as a vital part of the Waldeyer ring, is an important component of the immune system located centrally at the entrance of respiratory and gastrointestinal systems, where pathogens, infectious agents, and allergens invading the upper respiratory tract first encounter the immune system [1]. Tonsil tissue is a secondary lymphoid organ that exerts both humoral and cellular immunity functions against various antigens with specific antibodies, B and T cell activities, and protects the body especially in young children [2]. Hence, in patients with tonsillitis, cellular, and
humoral immune responses are stimulated together. Immunological reactions in tonsil tissue may give rise to chronic infections and hypertrophy. Chronic tonsillitis is the state of persistent inflammation associated with recurrent acute tonsillitis or subclinical infections. Tonsillar hypertrophy develops as a consequence of fibroid degeneration which starts in early childhood and continues until puberty and produces parenchymal hyperplasia or obstruction in crypts. Hypertrophy may also occur following bouts of local or systemic infection. However, they may also undergo atrophy with chronic disease processes [2].

Although chronic tonsillitis and tonsillar hypertrophy seem to have similar definitions and etiology, they are histopathologically distinct entities and their effect on immune cell compositions still remains to be understood. Though the cause of these two different changes in tonsil tissue is not completely clear, diet, genetics, and humoral changes may play role in etiology [3]. However, in some studies, it was reported that chronic tonsillitis or tonsillar hypertrophy result from the hypofunction of local and systemic immunity [4]. Nevertheless, there is a paucity of data on immune responses among these patients. Tonsil tissue removed surgically is an accessible source in order to examine the relationship between foreign pathogens, allergens, and the host immune systems. Tonsil tissue also provides a suitable in vivo model for investigating the inflammatory processes in lymphoid organs and infection mechanisms [5].

The Endoplasmic Reticulum (ER), which is an organelle with many important intracellular functions, is also critical for protein synthesis, folding and modification, and lipid synthesis, and calcium storage. ER synthesizes around one-third of the total proteome and plays a dominant role in the folding and maturation of proteins [6]. When the functional capacity of ER is exceeded or when ER functions become irregular, misfolded, or unfolded protein accumulation takes place in the ER lumen and this condition is termed as "ER stress" [7]. After ER stress develops in the cell, for the maintenance of vital functions of cells, a condition called [Unfolded Protein Response (UPR)], which reverses ER homeostasis, is triggered. UPR is characterized by different pathways inducing the survival or apoptosis of cells depending on the agent, intensity, and duration of ER stress, and the type of cell [6]. It was shown that the UPR response regulates intracellular metabolic, oxidative stress, and inflammation pathways [7].

The three main transmembrane proteins that occur as a result of the ER stress response and initiate the UPR signal are Activating transcription factor 6 (ATF6), protein kinase R-like ER kinase (PERK), and enzyme 1 (IRE1) that requires inositol [8]. These are normally in an inactive state. If ER stress is triggered by any pathology, they become active by separating from GRP78/binding protein (BIP), the main regulatory chaperon of UPR.

In particular, ATF6 induces the expression of transcription factors that regulate UPR elements after displacement to the nucleus and stabilizes both ER-associated protein degradation (ERAD) and ER-chaperones protein folding to reduce unfolded protein accumulation [9].

The active form of PERK phosphorylates both Eukaryotic translation initiation factor 2 (eIF2), which is involved in protein translation of ATF4 and induces protein folding factor gene expression to regulate ER homeostasis. IRE1 is the most conserved modulator of the ER stress signal pathway. However, if ER stress persists and UPR cannot provide hemostasis, apoptotic pathways are induced [10]. The first key molecule for ER stress-mediated apoptosis is the C/EBP homologous protein (CHOP), which increases cellular reactive oxygen species (ROS) production and Ca2+ concentration. Thus, the CHOP protein reduces the antiapoptotic expression of both B-cell lymphoma 2 (Bcl-2) and Bcl-2-associated X protein (Bax) [11].

The direct relation between ER stress and immunity responses is clear but as the casual relation between ER stress and immunity remains undetermined, this issue still attracts attention. In addition, mechanisms coordinating UPR signaling cascades with immunity still remains to be elucidated. Further studies are required in order to define the role of UPR in relevant regulatory mechanisms, signal molecules, and immunity. It was demonstrated that ER stress contributes significantly to the pathogenesis of various diseases including cancer, neurodegenerative disorders, inflammatory and autoimmune diseases, expression of inflammatory cytokines, and induction of inflammatory responses and enhances their severity [6]. If chronic or intense ER stress is not resolved, UPR induces apoptosis [7]. Tonsil tissue inhibits immune response in cases of chronic inflammation. However, whether inhibition of immune responses occurs via ER stress and UPR has not been examined so far. ER stress response in the immune system was investigated only in intestines up to date [12].

The present study aimed to investigate the role of ER stress response in molecular pathophysiological processes of chronic tonsillitis and tonsillar hypertrophy, two different pathologic entities, which occur in tonsil tissue where differentiation and maturation of immune cells take place and to contribute to the development of new treatment strategies targeting UPR pathways.

**Primary outcome**

Our primary outcome was to investigate whether ER stress response and apoptosis play a role in the etiopathogenesis of chronic tonsillitis and tonsillar hypertrophy, which are tonsil tissue diseases that play an important role in the functioning of the immune system of children.
Materials and methods

Study population, sample collection, and protocol

Our study was conducted on 46 patients (25 males, 21 females) aged between 2 and 16 years (mean age 6.2), who were clinically diagnosed with chronic tonsillitis and tonsillar hypertrophy by the Ear Nose Throat clinician. Patients with a history of immunodeficiency or systemic disease were excluded from the study. The tonsillectomy procedure was performed under general anesthesia using the classical dissection method. After the palatal pharyngeal archus mucosa was cut with low-heat plasma and the tonsil capsule was opened, a cryogenic plasma knife was used to remove the tonsil tissue and stop bleeding. Extracted tonsil tissue was classified as 1, 2, 3, and 4 using the Friedman staging system according to their size [13].

Chronic tonsillitis group (n = 23)

Patients with tonsil sizes grade 1 and 2, and tonsillectomy indications included frequently recurrent tonsillar infection, sore throat, and malodorous mouth problems were accepted as the chronic tonsillitis group.

Tonsillar hypertrophy group (n = 23)

Patients with tonsil size grades 3 and 4 and tonsillectomy indications included obstructive symptoms such as snoring, open mouth breathing, difficulty in breathing, and swallowing problems were accepted as the tonsillar hypertrophy group.

Measurements

Tonsillar hypertrophy and chronic tonsillitis groups were compared in terms of expression levels of genes in the ER stress pathway (ATF4, ATF6, EDEM1, CHOP, GRP78, EIF2AK3, ERN1, GRP94) and apoptosis pathway (BAX, BCL-2).

Real-time PCR analysis

Total RNA extraction from tissue samples obtained from patients was performed using TRizol® reagent (Invitrogen, Waltham, USA) according to the protocol previously described in the literature [14]. Tissue pieces were dissected by freezing in liquid nitrogen before extraction. cDNA synthesis was performed using the Transcriptor High-Fidelity cDNA Synthesis kit (Roche, Basel, Switzerland) using oligo (dT) and random primers, following the manufacturer’s instructions. Oligonucleotide primers were designed using the IDT DNA primer request tool and are manufactured by Biomers Inc. (Ulm, Germany). Primer sequences are given below. All PCR reactions were performed using LightCycler® 480 Instrument II (Roche, Penzberg, Germany) Real-time PCR via SYBR Green Master Mix (Bio-Rad Hercules, California, USA). Before proceeding with the qPCR process of the genes of interest, optimization processes of the primers used were carried out. Housekeeping genes such as 18S rRNA (18S ribosomal RNA), 28S rRNA (28S ribosomal RNA), ACTB (β-actin), β2M (β2-microglobulin), GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were examined for normalization. ACTB levels of these were found to be more stable, and studies continued with them. ΔCT results were normalized by ACTB. Expression results were calculated by the 2ΔΔct method. Primer sequences were shown in Table 1.

Western blot analysis

Antibodies and Western blot ATF-6 (D4B8) rabbit anti-human monoclonal (# 65,880), ATF-4 (D4B8) rabbit anti-human monoclonal (# 11,815), GRP78 (C50B12) rabbit anti-human monoclonal mAb (# 3177), CHOP (D46F1) rabbit anti-human monoclonal antibody (# 5554), PERK (C33E10) rabbit antihuman mAb (#3192), ERN1 (IRE1α) (14C10) rabbit antihuman mAb (#3294), Grp94 (D6X2Q) XP® rabbit antihuman mAb (#20,292), Bax (D2E11) rabbit antihuman mAb (#5023), Bcl-2 (D55G8) rabbit Antihuman mAb (#4223) and β-Actin (13E5) rabbit antihuman monoclonal antibody (# 4970) were obtained from cell signaling technology company (cell signaling tec., Leiden, Netherlands). Western blot analyzes were performed according to protocols previously described in the literature [15]. ACTB levels were used for normalization in Western blot analysis. For each group normalization, their own ACTB values were taken into account in this calculation. Changes in protein levels were determined after this normalization.
Statistical analysis

PCR primers were designed using IDT PrimerQuest software. Image-based data were analyzed by ImageJ software. The statistical significance was investigated using GraphPad Prism V6 software (GraphPad software Inc., La Jolla, USA). Expression results were calculated by the 2ΔΔct method. CT results were normalized by ACTB. All the experiments were performed as minimum triplicate (N ≥ 3). The SPSS version 22.0 (IBM Statistics) was used to analyze the data of the study. First of all, the conditions of the normal distribution of the data were examined with skewness and kurtosis values (values between 0.846 and 0.924) and Q-Q plot graphics, and it was observed that the data showed a normal distribution. Based on these results, an independent group t-test was used to compare the ER stress and apoptosis gene levels of chronic tonsillitis and tonsillar hypertrophy groups. The level of significance was set to p < 0.05.

Results

In the chronic tonsillitis group, the ΔCT levels of all ER stress genes except EDEM1 were found greater than the tonsillar hypertrophy group. These levels were considered as statistically significant (p < 0.005). And also, there was a significant difference between groups in ΔCT levels of apoptosis genes i.e., BAX, BCL-2 (p < 0.005). But, the apoptosis pathway ΔCT levels of the tonsillar hypertrophy group were significantly greater than the chronic tonsillitis group in contrast to ER stress ΔCT levels. Table 2, Fig. 1.

And also, we wanted to verify the results that we detected with the Real-time PCR method by using the Western blot method. According to the Western blot analysis, the

Statistical analysis

| Table 1 | Primers information used in the present study |
|---------|---------------------------------------------|
| Gene    | Sequence                                    |
| EDEM1   | Forward primer: CGGACGAGTACGAGAAGCCG | Reverse primer: CGTAGCAGAAGCCAGAATGC |
| ATF4    | Forward primer: ATGACCGAATGAGTTCTCTT | Reverse primer: GCTGAGAACCCTGAGGT |
| ATF6    | Forward primer: TCCTCGGTCAGTGAGCTCTTA | Reverse primer: CTTGGGCTGATTAGAAGTTTGT |
| GRP78   | Forward primer: CATCACCCGTCTCATTGCG | Reverse primer: CTGTTGACCCCTACTTCCT |
| CHOP    | Forward primer: GGAACAGATGTCATTCCC | Reverse primer: CTGCTTGACCCCTACTTCCT |
| EIF2AK3 (PERK) | Forward primer: GGAACACGAGCCGATTTTTT | Reverse primer: ACTATGTCATTATTGCAGCTTC |
| ERN1    | Forward primer: CACAGTGACGCTTCTGGAAC | Reverse primer: GCCATCATTAGATCTGGAGA |
| GRP94   | Forward primer: GCTGACGATGAGTTGATGAGG | Reverse primer: CATCGCTTCTGATCTTCTTATA |
| BAX     | Forward primer: CCGAGAGGTCTTTTTCGAG | Reverse primer: CCAGCCCATGATTGGCTGATT |
| BCL2    | Forward primer: GGTGGGTTCTATGTTGTTGG | Reverse primer: CGGTTCAGTACTGATCATCC |

| Table 2 | Comparison between tonsillar hypertrophy and chronic tonsillitis groups in terms of ER stress and apoptosis pathway markers |
|---------|----------------------------------------------------------------------------------------------------------------|
|         | Tonsillar hypertrophy (n = 32) | Chronic tonsillitis (n = 32) | t     | p    |
|         | Mean      | SD        | Mean      | SD        |       |     |
| ER stress pathway |
| ATF4 ΔCT | 7.01      | 2.18      | 9.41      | 1.11      | 5.540 | 0.000* |
| ATF6 ΔCT | 2.49      | 1.33      | 6.02      | 1.38      | 10.453 | 0.000* |
| EDEM1 ΔCT | 3.35      | 2.49      | 4.16      | 1.70      | 1.758 | 0.134** |
| CHOP ΔCT | −2.56     | 1.67      | −0.46     | 3.07      | 3.387 | 0.001* |
| GRP78 ΔCT | 1.87      | 2.01      | 4.68      | 1.80      | 5.898 | 0.000* |
| EIF2AK3 ΔCT | −3.03    | 2.19      | −1.23     | 1.77      | 3.610 | 0.000* |
| ERN1 ΔCT | 1.86      | 2.68      | 4.50      | 2.31      | 4.229 | 0.000* |
| GRP94 ΔCT | −1.96     | 2.04      | −0.25     | 1.94      | 3.429 | 0.000* |
| Apoptosis pathway |
| BAX ΔCT | 5.04      | 1.47      | 3.12      | 0.90      | 6.302 | 0.000* |
| BCL-2 ΔCT | 4.60      | 1.84      | 2.76      | 1.96      | 3.879 | 0.000* |

*p < 0.005; **p > 0.05
normalized protein levels of ATF4, ATF6, CHOP, GRP78, and ERN1 genes were found in the chronic tonsillitis group 2.63, 7.56, 2.28, 2.34, and 2.41 times greater, respectively, compared to the tonsillar hypertrophy group. These increases were considered significant. There was no significant difference in the levels of the apoptosis-associated BAX, BCL-2 genes in terms of Western blot analysis. Figure 2.

Discussion

The most common indications of tonsillectomy, which is one of the most common operations performed in children all over the world, are chronic tonsillitis and tonsillar hypertrophy. It was demonstrated that immunological parameters, genetic susceptibility, and local lymphocyte dysfunction play role in the etiology of chronic tonsillitis and tonsillar hypertrophy, which share common histological properties [16]. In this study, it was established that ER stress response and apoptosis pathway also play an important role in the pathogenesis of chronic tonsillitis and tonsillar hypertrophy.

When dysfunction occurs in the ER, all cells are programmed to respond to it. However, when the ER stress process is prolonged and intensified, the proapoptotic process becomes dominant and ER stress can cause cell apoptosis [17]. The role of ER stress, an important mechanism mediating apoptosis, has not been investigated in the etiopathogenesis of chronic tonsillitis and tonsillar hypertrophy. In a previous study in which tonsillar hypertrophy and recurrent tonsillitis were compared, it was concluded that apoptosis has a role in the pathogenesis of tonsillar hypertrophy [16].

Apoptosis is actually an indicator of proliferative activity. In other words, in cases where cellular proliferation is high such as tonsillar hypertrophy, an increase in cellular apoptosis would be expected to limit cell proliferation. According to the results of the real-time PCR of our study, the ΔCT levels of apoptosis proteins in the tonsillar hypertrophy group were found to be greater than in the chronic tonsillitis group.

As it is known, tonsil and adenoid tissues have an important role in the development of immunity in childhood [16, 18]. The tonsils are a part of the mucosa-associated lymphoid tissue and are considered as secondary lymphoid
organs where foreign allergens, viruses and bacteria entering the body through the respiratory or gastrointestinal tract are captured [19]. However, according to the results of a recent study that seems to contradict this information, allergic rhinitis and bronchial asthma symptoms improved after tonsillectomy in pediatric patients who underwent tonsillectomy due to tonsillar hypertrophy and recurrent tonsillitis, while symptoms did not improve in patients who did not undergo tonsillectomy [20]. In the same study, the incidence of fever after tonsillectomy was significantly reduced in patients with recurrent infections. These results were attributed to the negative role of tonsillar tissue in the immune defense response to infection in cases of tonsillar hypertrophy and recurrent tonsillitis. The fact that the ER stress response in patients with chronic tonsillitis was greater than in patients with tonsillar hypertrophy and the apoptosis level in patients with tonsillar hypertrophy was greater compared to patients with chronic tonsillitis in our study may explain this contradiction. On the other hand, apoptosis also occurs when there is an increase in lymphocytes secondary to recurrent infections such as chronic tonsillitis. In this case, apoptosis is thought to control lymphocyte increase and cellular hyperplasia [16]. The absence of significant difference between the two groups in terms of apoptosis genes according to Western blot analysis in contrast to Real-time PCR may be due to the occurrence of apoptosis in both pathological conditions.

Ji et al., [21] examined the effect of e-cigarette aerosols on normal human keratocytes and the role of ER stress in this process was detected via the increase in the expression of CHOP, ATF4, XBP1, and IRE1α genes. In a human study investigating the effect of ER stress and apoptosis on osteolysis, ER stress and apoptosis were evaluated to cause loosening and loss of the prosthesis, and the apoptosis expression genes (BCL-2 and BAX) and ER stress markers (IRE1-α, GRP78 / Bip, CHOP, cleaved caspase-4, and JNK) expression levels were found to be significantly increased compared to the control group and there was a correlation between the degree of ER stress and the clinical severity of osteolysis. Thus, it was proven that ER stress exerts an impact on the apoptotic process in osteolysis [22]. In the present study, higher expression of GRP78, EDEM1, ATF6, and ATF4, which are ER stress markers, was observed in chronic tonsillitis groups than in the tonsillar hypertrophy group. And also, CHOP was up-regulated in the chronic tonsillitis group, as a pro-apoptotic protein, but a slightly higher apoptosis level was found in the tonsillar hypertrophy group. Moreover, CHOP down-regulates the expression of BCL-2, sensitizing cells to apoptosis. The relationship between ER stress-induced apoptosis and tonsillar diseases should be further explored.

Studies on human epithelial cells have investigated the role of ER stress in the development of epithelial-to-mesenchymal transition, in multiple tissues, including the eye, lungs, liver, and kidneys, and it has been revealed that ER stress triggers the development of epithelial-to-mesenchymal transition and causes pathological conditions.
The role of ER stress, which is an unfolded protein response, in the development of organ fibrosis is accepted. It was determined that ER stress played role in peritoneal fibrosis and apoptosis and that preconditioning produced by ER stress may prevent fibrosis in patients having peritoneal fibrosis [23].

In a study evaluating the antitumorigenic effects of Arachidonic acid (AA) in HT-29 human colon cancer cells, the resulting ER stress in HT-29 cells treated with AA was demonstrated by an increase in the level of the processed form of XBP1 and phosphorylated eIF2α [24]. The resultant ER stress and induced apoptosis were thought to result from the provision of more AA to membrane lipids and altered membrane properties of the ER. The molecular mechanism of the anti-cancer activity of DWP05195, a novel transient receptor potential vanilloid 1 (TRPV1) antagonist, was investigated in human ovarian cancer cells. DWP05195 has been shown to induce apoptosis by inducing (CHOP) expression and ER stress. In the same study, with the ER-stress inhibitor 4-PBA, DWP5195-induced cell death was significantly suppressed [25].

In another study to confirm whether ER stress contributes to Mn-induced cell death, pretreatment with the ER stress inhibitor 4-PBA has been shown to reduce Mn-induced neurotoxicity. Significant increase in the expression of GRP78, PERK, eIF2α and ATF4 proteins in ER stress signaling pathways with Mn incubation showed that Mn-induced neurotoxicity occurred through the ER stress pathway. It was also shown in the same study that ER stress induces CHOP and caspase-12 mediated apoptosis [26]. Phosphorylated eIF2α signaling can trigger cell death depended on induce of CHOP expression. As our results showed, no significant difference was found in the expression of total PERK protein and EIF2AK3 gene between the two groups according to the Western blot analysis. The detection of phosphorylated eIF2α can help to know the activation of ER stress-related apoptosis.

Conclusion

According to our study, although it can be said that ER stress may play role in the pathogenesis of chronic tonsillitis, and apoptosis may play role in the pathogenesis of tonsillar hypertrophy, it can not account for the pathogenesis of this complex process on its own. Information regarding the pathogenesis of tonsil diseases is very limited. It is necessary to carry out new studies on this subject both to reveal the pathogenesis of tonsil diseases and to develop alternative treatments, especially for disorders related to the immune system.

Acknowledgements This work was supported by the Selcuk University Scientific Research Projects Coordination Unit under Grant number 19401029. We would like to express our gratitude to Prof. Neslihan Saltalı from Ordu University for her help in the statistical analysis part.

Authors’ contributions Conceiving and designing the study; or collecting the data; or analyzing and interpreting the data; MO, NK, FD, CE, OE, OO, writing the manuscript or providing critical revisions that are important for the intellectual content; MO, OO, MKB approving the final version of the manuscript; MO, MKB, CE, OE, Relationship of endoplasmic reticulum stress with the etiopathogenesis of chronic tonsillitis and tonsillar hypertrophy in pediatric patients: a prospective, parallel-group study.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Selcuk University Faculty of Medicine Clinical Research Ethics Committee) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethics approval for the study was obtained from the Clinical Research Ethics Committee of Selcuk University Medical Faculty (Ref No: 2018/329). Our study was supported by the Selcuk University Scientific Research Projects Coordination Unit (Project Number: 19401029). After obtaining informed consent from the parents of the patients to be included in the study, we conducted our study by the ethical standards stated in the 1964 Helsinki Declaration. Human tonsil samples used in this study were obtained from tonsillectomy patients in Selcuk University, Faculty of Medicine Hospital, Department of Otolaryngology between the dates of November 2018 and August 2019.

Consent to participate Informed consent was obtained from all patients’ parents.

References

1. Ogra PL (2000) Mucosal immune response in the ear, nose and throat. Pediatr Infect Dis J 19(suppl):S4–S8. https://doi.org/10.1097/00006454-200005010-00002
2. Yan Y, Song Y, Liu Y, Su J, Cui L, Wang J, Geng J, Liu X, Shi Y, Quan S, Hang A, Zuo L (2019) Short and long-term impacts of adenooidectomy with/without tonsillectomy on immune function of young children <3 years of age A cohort study. Medicine (Baltimore) 98(19):e15530. https://doi.org/10.1097/MD.0000000000015530
3. Alan D, Kornblut A (1991) Non neoplastic diseases of the tonsils and adenoids. In: Paparella M, Shumrick DA, Gluckman JL, Meyerhof WL (eds) Otorhinolaryngology, vol 3, 3rd edn. W. B. Sounders Company, New York, pp 2129–47
4. Hata M, Asakura K, Saito H, Morimoto K, Kataura A (1996) Profile of immunoglobulin production in adenoid and tonsil lymphocytes. Acta Otolaryngol Suppl 523:84–86
5. Palomares O, Rückerl B, Jartti T, Kückeßzer UC, Puhakka T, Gomez E, Fahrner EB, Speiser A, Jung A, Kwok WK, Kalogjera L, Akdis M, Akdis CA (2012) Induction and maintenance of allergen-specific FOXP3+ Treg cells in human tonsils as potential first-line organs of oral tolerance. J Allergy Clin Immunol 129(2):510–520. https://doi.org/10.1016/j.jaci.2011.09.031

 Springer
6. So JS (2018) Roles of endoplasmic reticulum stress in immune responses. Mol Cells 41(8):705–716. https://doi.org/10.14348/molcells.2018.0241

7. González-Quíroz M, Blondel A, Sagredo A, Hetz C, Chevet E, Pedraux R (2020) When endoplasmic reticulum proteostasis meets the DNA damage response. Trends Cell Biol 30(11):881–891. https://doi.org/10.1016/j.tcb.2020.09.002

8. Amadio G, Molteo O, Fasano D, Zerillo L, Oliveti M, Di Pietro P, Faranoinio R, Barone P, Pellecchia MT, De Rose A (2019) PERK-mediated unfolded protein response activation and oxidative stress in PARK20 fibroblasts. Front Neurosci 27(13):673. https://doi.org/10.3389/fnins.2019.00673

9. Gardner BM, Walter P (2011) Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response. Science 333(6051):1891–1894. https://doi.org/10.1126/science.1209126

10. Urra H, Dufey E, Lisbona F, Rojas-Rivera D (1833) Hetz C (2013) When ER stress reaches a dead end. Biochim Biophys Acta 12:3507–3517. https://doi.org/10.1016/j.bbamcr.2013.07.024

11. Puthalakath H, Oeilly LA, Gunn P, Lee L, Kelly PN, Huntington ND, Hughes PD, Michalak EM, McKimm-Breschkin J, Motoyama N et al (2007) ER stress triggers apoptosis by activating BH3-only protein Bim. Cell 129(7):1337–49. https://doi.org/10.1016/j.cell.2007.04.027

12. Wang S, Kaufman RJ (2012) The impact of the unfolded protein response on human disease. J Cell Biol 197(7):857–67. https://doi.org/10.1083/jcb.201110131

13. Shin HS, Ryu ES, Oh ES, Kang DH (2015) Endoplasmic reticulum stress as a novel target to ameliorate epithelial-to-mesenchymal transition and apoptosis of human peritoneal mesothelial cells. Lab Invest 95(10):1157–1173. https://doi.org/10.1038/labinvest.2015.91

14. Bae S, Kim M-K, Kim HS, Moon Y-A (2020) Arachidonic acid induces ER stress and apoptosis in HT-29 human colon cancer cells. Anim Cells Syst (Seoul) 24(5):260–266. https://doi.org/10.1080/19768354.2020.1813805

15. Wang Y-Y, Lee K-T, Lim MC, Choi JH (2020) TRPV1 antagonist DWP05195 induces ER stress-dependent apoptosis through the ROS-p38-CHOP pathway in human ovarian cancer cells. Cancers 12(6):1702. https://doi.org/10.3390/cancers12061702

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.