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Significance of TNFSF11 and TNFRSF11B in Middle Ear Cholesteatoma of Children and Adults

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Abstract: Middle ear cholesteatoma is a common ear disease with different manifestations and pathological mechanisms in children and adults.Middle ear cholesteatoma is more severe in children than adults. We aimed to detect the expression of tumor necrosis factor ligand superfamily member 11 (TNFSF11) and tumor necrosis factor receptor superfamily member 11B (TNFRSF11B) and analyze the difference in ear bone destruction in middle ear cholesteatoma in children and adults.Through the comprehensive analysis of related studies, the mechanism of its action in the progression of the disease was expounded, and the theoretical basis for clinical treatment was provided. A total of 18 children and 32 adults with middle ear cholesteatomas were examined. The degree of bone destruction was observed. TNFSF11 and TNFRSF11B expressions in the cholesteatoma and normal external auditory canal skin were detected by immunohistochemistry. Bone destruction was more severe in children with middle ear cholesteatoma. TNFSF11 expression in cholesteatoma was significantly higher in children than adults, whereas expression in external auditory canal skin was not significantly different between groups. Expression of TNFRSF11B in cholesteatoma and external auditory canal skin was not significantly different between children and adults. In children and adults, TNFSF11 in cholesteatomas was not correlated with TNFRSF11B. TNFSF11 expression was positively correlated with the degree of ear bone destruction, unlike TNFRSF11B. TNFSF11 expression in children with cholesteatoma is higher than adults and is involved in the molecular biological mechanism underlying its destructive nature. These findings will help us develop better treatments.

Keywords: Cholesteatoma of the Middle Ear; Skin of the External Auditory Canal; Tumor Necrosis Factor Ligand Superfamily Member 11; Tumor Necrosis Factor Receptor Superfamily Member 11B; Degree of Ear Bone Destruction

1. Introduction

Cholesteatoma of the middle ear is a common clinical condition, and its main pathological features include bone destruction, which can lead to hearing loss, facial paralysis, labyrinthine fistula, and even intracranial complications. It has been suggested that middle ear cholesteatomas in children are less reparative than in adults, and the proportion of parabasal tissue was found to be greater in children with cholesteatomas than in adults with cholesteatomas [1]. The current treatment for children or adults with cholesteatoma is primarily surgical, and there is no stan-

dard treatment to cure or prevent recurrence of cholesteatoma [2]. Its clinical symptoms include otorrhea, hearing loss, dizziness, vertigo, facial paralysis, and even intracranial and extracranial complications. Clinically, the exact mechanism of acquired cholesteatoma formation is not clear, see the following ideas [3]: 1. squamous epithelial cell chemotaxis results; 2. pocket invagination results; 3. epithelial cell migration results; 4. basal cell proliferation results. The mechanism of bone destruction in cholesteatoma is described by two main theories, "mechanical stress theory" and "biochemical theory". According to the mechanical pressure theory, as the volume of keratin fragments in cholesteatoma tissues increases, a continuous accumulation of pressure is formed, which affects the local blood supply and stimulates an increase in osteoclast activity, leading to bone necrosis. However, the results of pathophysiological studies do not support the above view, suggesting that natural apoptosis, necrotic apoptosis, or cellular autophagy occur primarily as a result of reduced local self-regulation. Biochemical theory suggests that cholesteatoma tissue contains a large number of enzymes and inflammatory mediators that play an important role in bone destruction. The extracellular matrix interacts with the cholesteatoma itself, leading to the production of large amounts of protein hydrolyzing enzymes and inflammatory mediators, and the extracellular matrix is in close proximity to the temporal bone and auditory tubercle, leading to bone resorption and destruction.

More and more researchers are focusing on the role of cytokines in the development of cholesteatoma. A previous study has demonstrated that the destruction of bone by cholesteatoma is associated with levels of cytokines such as tumor necrosis factor ligand superfamily member 11 (TNFSF11) and tumor necrosis factor receptor superfamily member 11B (TNFRSF11B) [4]. Clinically, cholesteatoma is more aggressive in children than in adults, with more serious bone destruction, a wide range of lesions, and a high recurrence rate reported [5, 6]. At present, only a few studies have reported on this phenomenon, and it is necessary to study the mechanism underlying this phenomenon to guide the treatment of cholesteatoma in children. We hypothesized that the high content of TNFSF11 and the low content of TNFRSF11B are the reasons why cholesteatoma in children is more serious than that in adults. Therefore, this study aimed to conduct a comparison of the expression of TNFSF11 and TNFRSF11B in children and adults with cholesteatoma and to explore the molecular biological mechanism by which middle ear cholesteatoma is more destructive and invasive in children than in adults, with the objective of providing further evidence for the prevention and treatment of this disease.

2. Methods

Fifty patients with acquired secondary middle ear cholesteatoma, who were hospitalized in the XXXX Hospital (anonymized for review) from May 2018 to January 2021 were selected as study participants (Appendix A Table A1). All patients were treated with surgery for the first time in one ear and were categorized according to age into the children group (≤18 years) or the adult group (>18 years).

This study was conducted according to the tenets of the Helsinki Declaration of 1975, as revised in 2008. All patients or their family members provided written informed consent. The study was approved by the Ethics Committee of XXXX Hospital (No. XXXX) (anonymized for review). The study adhered to the STROBE guidelines for non-randomized public behavior.

The primary antibodies (rabbit anti-human TNFSF11 and TNFRSF11B polyclonal antibodies) were purchased from Abcam (Cambridge, UK), the secondary antibody (HRP-labelled sheep anti-rabbit IgG (H+L) kit) was purchased from EarthOx (San Francisco, USA), and the diaminobenzidine (DAB) color kit was purchased from Gene Technology Co., Ltd. (Shanghai, China).

According to computed tomography (CT) pre-judgment and intraoperative observation under a microscope (Carl Zeiss, Oberkochen, Germany), the destruction of ear bone was recorded as follows: degree I, only one auditory ossicle was damaged, or destruction of the auditory ossicle and shield plate was not obvious; degree II, two or three ossicles were damaged; degree III, the ossicles were completely destroyed, and the posterior wall of the external auditory canal, facial nerve canal, and inner ear had lesions [7].

Fresh cholesteatoma and normal external auditory canal skin samples on the same side of the affected ear were fixed with 4% paraformaldehyde, embedded in paraffin, and sliced into 3–4 μm thick continuous sections. The EnVision method was used for immunohistochemistry, involving: haematoxylin and eosin (HE) staining, dehydration, transparency, blocking endogenous peroxidase activity, and antigen repair. The primary antibodies (1:100 dilution) against TNFSF11 and TNFRSF11B were placed on the slices followed by incubation in a wet box for 1 hour and refrigeration at 4 ℃ overnight. The samples were washed with phosphate-buffered saline (PBS) to remove un-

bound primary antibodies. The secondary antibody was placed on the slices, followed by incubation in a 37 °C wet box for 30 minutes. The slices were stained with DAB staining solution, and then stained with haematoxylin and sealed with neutral gum. PBS replaced the primary antibody as the negative control, and a known positive sample was used as the positive control.

The percentage of positive cells was calculated by randomly selecting five non-overlapping areas under 200× magnification. The staining was judged as negative when the average percentage of positive cells was less than 10% [8].

IBM® SPSS® Statistics software (Armonk, NY, USA) was used to analyze and process the data. The ear bone destruction between different age groups was compared using the $\chi 2$ test, the rate of immunohistochemically positive cells was compared using the t-test, and the correlation between the positive expression of TNFSF11 and TNFRSF11B and the degree of bone destruction was compared using multiple analysis of variance (least significant difference t-test).

3. Results

A total of 18 children and 32 adults with middle ear cholesteatomas were examined. No significant differences were noted between the two groups in terms of sex, degree of mastoid gasification, and course of disease.

3.1. Degree of Bone Destruction

The degree of ear bone destruction was more serious in children than in adults (Table 1, Figures 1 and 2).

Table 1. Ear bone destruction in children and adults with middle ear cholesteatoma (patients, %).

Group	Patients	Degree I Linear Measure	Degree II Linear Measure	Degree III Linear Measure	X^2	*P*
Children group	18	3 (16.7)	6 (33.3)	9 (50.0)	3.840	0.039
Adult	32	12 (37.5)	12 (37.5)	8 (25.0)		

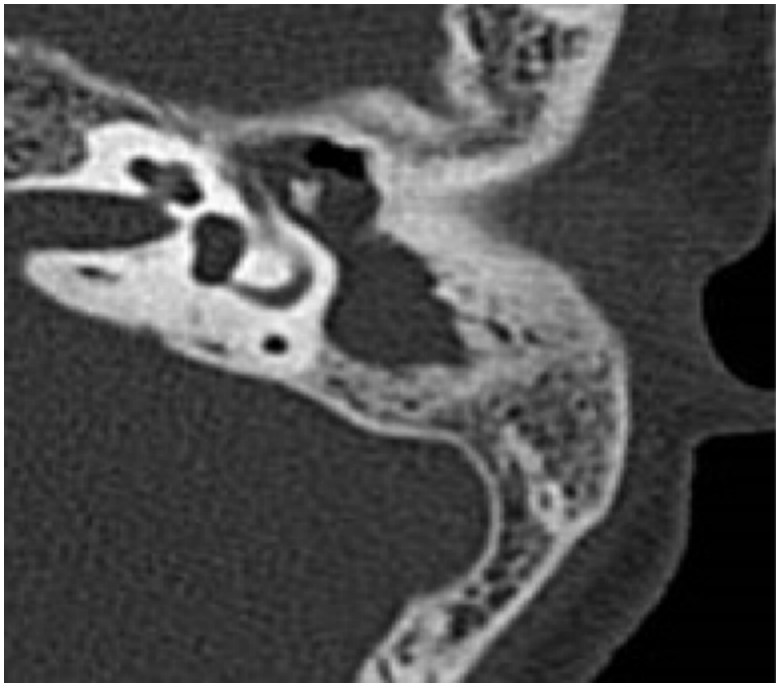

Figure 1. Computed tomography of temporal bone in a child (There was much cholesteatoma in the middle ear, extensive bone destruction, and blurred ossicles).

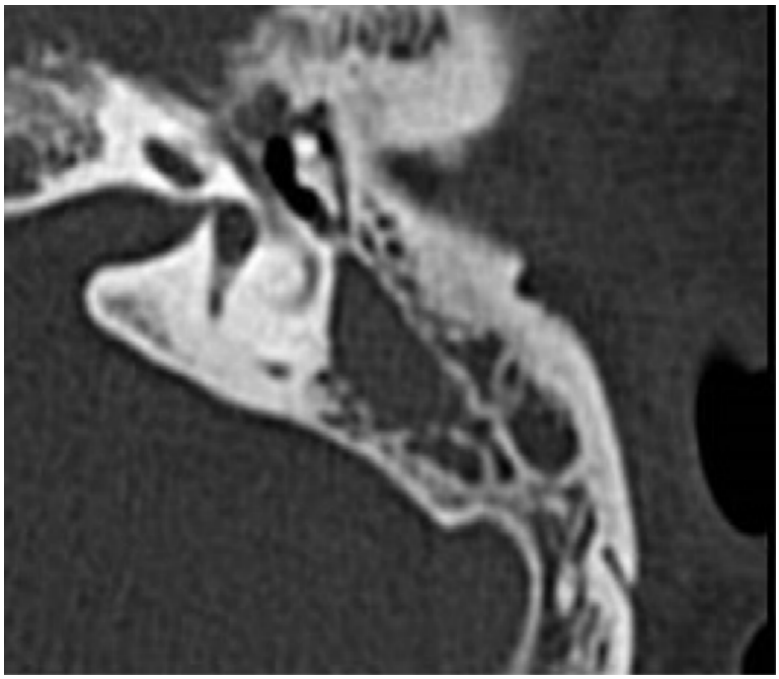

Figure 2. Computed tomography of temporal bone in an adult (Middle ear cholesteatoma was less, bone destruction was light, and ossicles were clear).

3.2. Expression of TNFSF11 and TNFRSF11B in Cholesteatoma

The positive expression of TNFSF11 and TNFRSF11B in cholesteatoma was mainly distributed in the nucleus of the whole epithelial layer and subepithelial tissue. Some expression was also noted in the cytoplasm, with brownish yellow staining (Figures 3–6).

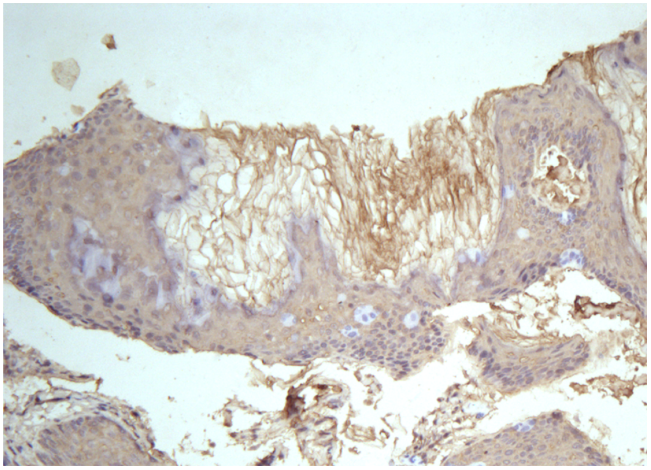

Figure 3. Expression of TNFSF11 in cholesteatoma of a child (SP ×200) (There were many positive cells with deep staining, and both the nucleus and cytoplasm were stained).

Statistical analysis revealed that the expression of TNFSF11 was significantly higher in children with cholesteatoma than in adults, but the expression of TNFRSF11B was not significantly different between the two groups (Table 2).

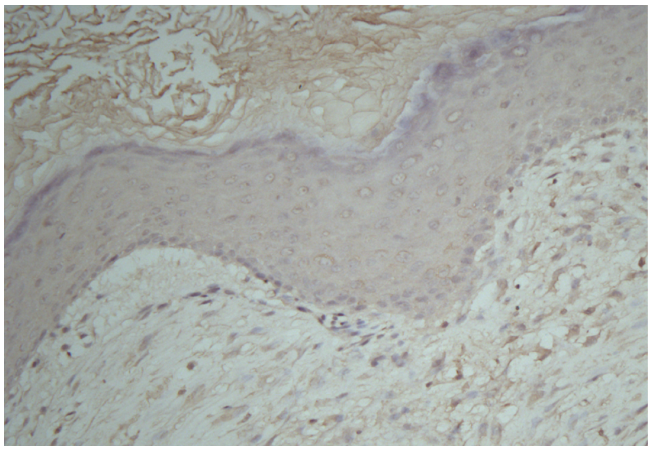

Figure 4. Expression of TNFSF11 in cholesteatoma of an adult (SP ×200) (The positive cells were few, and the staining was shallow and mainly located in the nucleus).

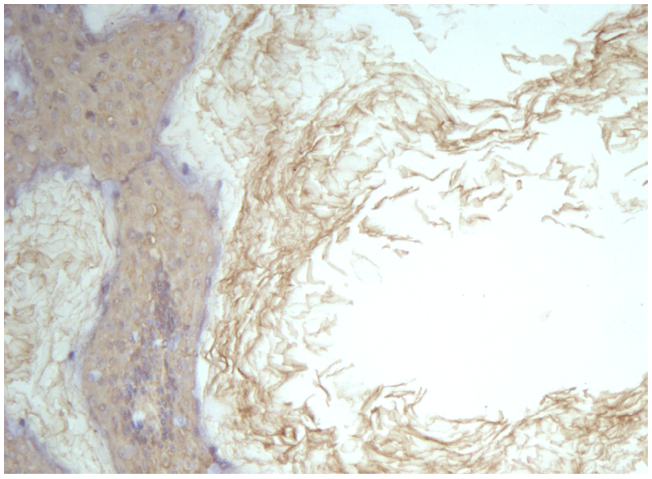

Figure 5. Expression of TNFRSF11B in cholesteatoma of a child (SP ×200) (The positive cells were few, the staining was shallow, and the staining was mainly located in the nucleus).

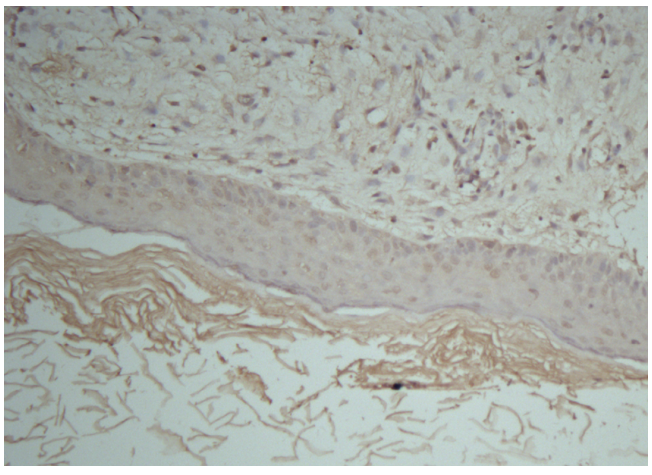

Figure 6. Expression of TNFRSF11B in cholesteatoma of an adult (SP ×200) (The positive cells were few, the staining was shallow, and the staining was mainly located in the nucleus).

Table 2. Positive expression of TNFSF11 and TNFRSF11B in children and adults with middle ear cholesteatoma (patients, %).

	Group	Patients	Percentage of Positive Cells	*t*	*P*
TNFSF11	Children group	18	88.97 ± 13.40	2.17	0.017
	Adult group	32	65.69 ± 10.82		
TNFRSF11B	Children group	18	55.61 ± 11.36	1.51	0.329
	Adult group	32	53.16 ± 9.08		

Note: TNFSF11, tumor necrosis factor ligand superfamily member 11; TNFRSF11B, tumor necrosis factor receptor superfamily member 11B.

3.3. Expression of TNFSF11 and TNFRSF11B in the Skin of the Normal External Auditory Canal

The positive expression of TNFSF11 and TNFRSF11B in the skin of the external auditory canal was located in the nucleus of the epidermal layer and the subcutaneous connective tissue, and some expression was also noted in parts of the cytoplasm, with brown-yellow staining (Figures 7–10).

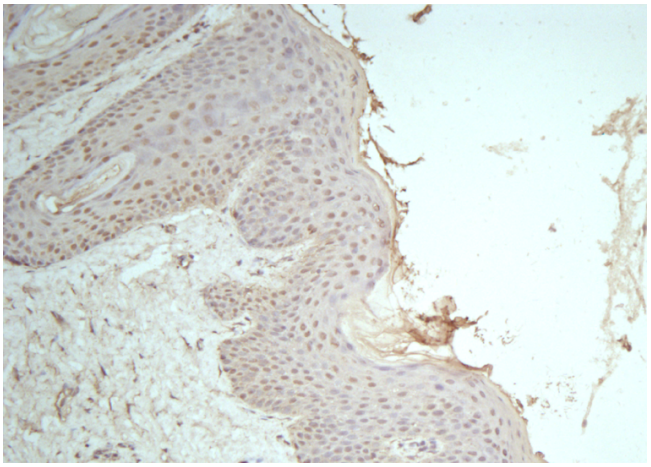

Figure 7. Expression of TNFSF11 in the external auditory canal skin of a child (SP ×200) (There were few positive cells with light staining, and the staining was mainly located in the nucleus).

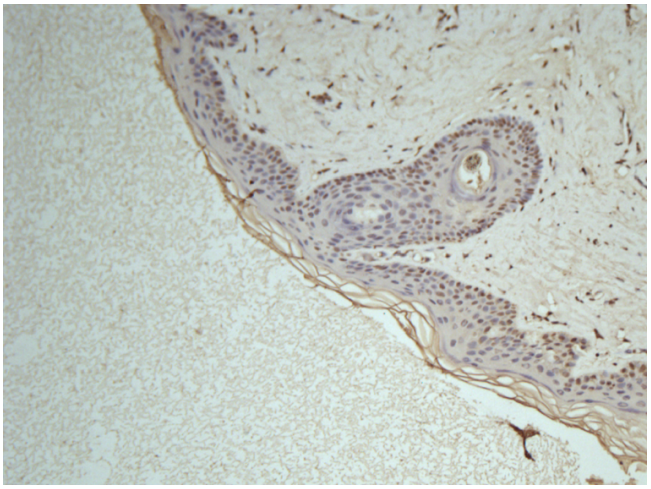

Figure 8. Expression of TNFSF11 in the external auditory canal skin of an adult (SP ×200) (There were few positive cells with light staining, and the staining was mainly located in the nucleus).

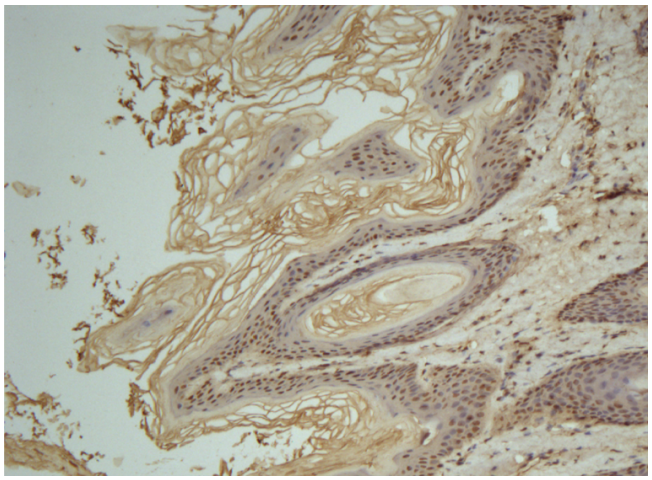

Figure 9. Expression of TNFRSF11B in the external auditory canal skin of a child (SP ×200) (The positive cells were few, the staining was shallow, and the staining was mainly located in the nucleus).

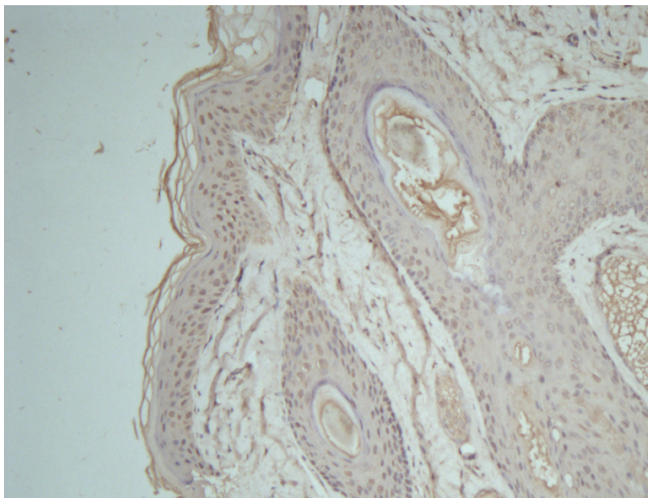

Figure 10. Expression of TNFRSF11B in the external auditory canal skin of an adult (SP ×200) (The positive cells were few, the staining was shallow, and the staining was mainly located in the nucleus).

Statistical analysis revealed no significant difference in the expression of TNFSF11 and TNFRSF11B in the external auditory canal skin among patients in different age groups (Table 3).

Table 3. Positive expression of TNFSF11 and TNFRSF11B in the external auditory canal skin of children and adults (cases, %).

	Group	Case	Percentage of Positive Cells	*t*	*P*
TNFSF11	Children group Adult group	18 32	38.89 ± 6.71 37.50 ± 6.52	2.13	>0.05
TNFRSF11B	Children group Adult group	18 32	61.11 ± 10.21 53.13 ± 9.07	1.57	>0.05

Note: TNFSF11, tumor necrosis factor ligand superfamily member 11; TNFRSF11B, tumor necrosis factor receptor superfamily member 11B.

3.4. Correlation Analysis of TNFSF11 and TNFRSF11B

No significant correlation was noted between the expression of TNFSF11 and TNFRSF11B in middle ear cholesteatoma in children or adults (Table 4).

Table 4. Correlation between TNFSF11 and TNFRSF11B in children and adults with cholesteatoma.

Group	TNFSF11	TNFRSF11B		Total	*r*	*P*
		Positive	Negative			
Children	positive	8	7	15	0.100	1.008
	negative	2	1	3		
	total	10	8	18		
Adult	positive	10	8	18	0.055	0.871
	negative	9	5	14		
	total	19	13	32		

Note: TNFSF11, tumor necrosis factor ligand superfamily member 11; TNFRSF11B, tumor necrosis factor receptor superfamily member 11B.

3.5. Correlation Analysis of the Expression of TNFSF11 and TNFRSF11B and Ear Bone Destruction in Cholesteatoma

3.5.1. Correlation Analysis of TNFSF11 Expression and Ear Bone Destruction

The expression of TNFSF11 was positively correlated with the degree of ear bone destruction in both children and adults (Table 5).

Table 5. Correlation between TNFSF11 expression and bone destruction in middle ear cholesteatoma in children and adults.

Group	Ear Bone Destruction Classification (Patients)	Percentage of TNFSF11-Positive Cells (%)	*t*	*P*
Children	Degree I (3)	79.39 ± 11.81	2.562	0.019
	Degree II (6)	87.90 ± 12.77		
	Degree III (9)	93.74 ± 15.02		
Adult	Degree I (12)	55.18 ± 10.15	2.391	0.027
	Degree II (12)	64.06 ± 11.57		
	Degree III (8)	74.73 ± 12.90		

Note: TNFSF11, tumor necrosis factor ligand superfamily member 11.

3.5.2. Correlation Analysis of TNFRSF11B Expression and Ear Bone Destruction

No significant correlation was noted between the expression of TNFRSF11B and the degree of ear bone destruction in either children or adults with middle ear cholesteatoma (Table 6).

Table 6. Correlation between tumor necrosis factor receptor superfamily member 11B (TNFRSF11B) expression and bone destruction in middle ear cholesteatoma in children and adults.

Group	Ear Bone Destruction Classification (Patients)	Percentage of TNFRSF11B-Positive Cells (%)	*t*	*P*
Children	Degree I (3)	48.07 ± 9.88	0.906	0.302
	Degree II (6)	53.34 ± 10.97		
	Degree III (9)	58.06 ± 13.60		
Adult	Degree I (12)	48.97 ± 10.03	1.411	0.148
	Degree II (12)	53.99 ± 11.95		
	Degree III (8)	57.28 ± 13.09		

4. Discussion

In this study, the content of TNFSF11 and TNFRSF11B, the degree of bone destruction, and their correlation between children and adults with cholesteatoma were studied. The content of TNFSF11 in children with cholesteatoma was found to be higher than that in adults, causing more serious ear bone destruction, which may be an important reason why children with cholesteatoma experience more harm than adults.

Based on the national and international literature reports, we have gained insight into the fact that cholesteatoma of the middle ear is a disease of key concern in the Department of Otorhinolaryngology. Through the efforts of scholars from various countries to drill down, we found that keratinized squamous epithelial cells within the temporal bone are highly proliferative and are included in the cholesteatoma pathogenesis [9]. Cholesteatoma is a type of benign lesion of the squamous epithelium. Although it is not a true tumor, its biological characteristics are similar to those of a tumor and it has the ability to invade and destroy local bone tissue. The pathogenesis of cholesteatoma and the factors that influence it are complex and varied [10]. Histologically, cholesteatoma tissue is similar to skin tissue, and hyperproliferation of cholesteatoma keratinized squamous epithelial cells can be distinguished from skin [11]. Microscopically, the lesion is divided into 3 layers: cholesteatoma has three basic features: epithelial tissue (composed of highly proliferative squamous epithelium), subepithelial connective tissue (presence of inflammatory cells), and capsule contents. The basal, stratum spinosum, stratum granulosum, and stratum pellucidum constitute the epithelial tissue, and the capsule contents include purulent necrotic material and differentiated keratinous scales [12]. Localized accumulation of hyperproliferative keratinized squamous epithelium is one of the main features of cholesteatoma of the middle ear. At present, scholars at home and abroad have done a lot of research work on the characteristics of cholesteatoma epithelium, bone destruction and resorption, and the accumulation of keratin debris, which has brought the study of the etiology and pathogenesis of cholesteatoma-type otitis media to a new field. Abnormal immune response is an intrinsically important factor in the persistent development of cholesteatoma and bone destruction, and a variety of inflammatory factors play an important role in mediating this abnormal immune response. More and more inflammatory factors have been shown to be abnormally expressed in cholesteatoma, such as interleukins 1, 6, and 8, tumor necrosis factor, epidermal growth factor, fibroblast growth factor, etc., and it is believed that all of these inflammatory factors are responsible for the development of cholesteatoma. These inflammatory factors are all important contributors to the proliferative apoptosis of the cholesteatoma epithelium and the accumulation of resorbed keratinous debris by bone destruction. The rapid development of molecular biology technology has provided advanced technical detection means to explore the pathogenesis and mechanism of middle ear cholesteatoma; over the years, scholars at home and abroad have carried out extensive research on the pathogenesis and mechanism of middle ear cholesteatoma, mainly focusing on the proliferation of cholesteatoma epithelial cells, the erosive behavior of the bone, and the apoptosis of keratocytes and other aspects of the research. Bassiouny, Badour and Omran [13] proposed that the matrix tissue of adult cholesteatoma tissue was significantly lower than that of children, further explaining the more aggressive and less repair of pediatric cholesteatoma.

This study observed more severe ear bone destruction in children with middle ear cholesteatoma than in adults, which is consistent with the findings of Jackson, Addison and Prinsley [5] and Kalia et al. [6]. Some studies reporting on the reasons why bone destruction in middle ear cholesteatoma is more serious in children than in adults have demonstrated no significant structural difference between children and adult middle ear cholesteatoma [13]. A previous study has stated that the repair in children was worse than that in adults, and the proportion of stromal tissue was greater in children than that in adults [1]; however, more research has focused on osteoblasts and osteoclasts (OCs). Bone morphogenesis, reconstruction and destruction are fundamental biological processes that occur under a variety of pathophysiological conditions. It is a dynamic equilibrium process, and whether it is based on reconstruction or destruction depends on the relative magnitude of the interaction between OCs and osteoblasts. In recent years, the major activation pathway of OCs has been found to be the RANKL/RANK/OPG pathway, and RANKL is a member of the tumor necrosis factor (TNF) ligand family. Osteoblasts and OCs play an important role in the breakdown and absorption of bone by cholesteatoma, and the OC is the final acting cell for bone destruction in cholesteatoma. TNFSF11 (tumor necrosis factor (ligand) superfamily member 11), also known as OPGL (Osteoprotegerin Ligand) and RANKL (Receptor activator of NF-κ B ligand), is a secreted glycoprotein discovered by Simonet et al. [14] in 1997. It is a tumor necrosis factor receptor superfamily member, secreted by the evolved cells of a

variety of mesenchymal cells, such as osteoblasts, bone marrow stromal cells, endothelial cells, Vascular smooth muscle cells, etc. [15]. In 1998, Lacey et al. [16] used the OPG fusion protein needle studies to identify myeloid mononuclear cell lines with OPG binding sites on the cell surface and separate mononuclear thin lines from mouse bone marrow This ligand (OPGL) was cloned into the cDNA library of cell and human lymph nodes. In the same year, OPG/OCIF ligands were also cloned in the cDNA library of mouse bone marrow stroma-derived ST2 cells [17]. This ligand is a membrane-bound protein, a member of the TNF ligand family, that induces osteoclast-like cell formation, but this effect is rescued by OPG/OCIF. Bone resorption-stimulating factor upregulates the expression of this protein. This is consistent with the previously hypothesized biological profile of osteoclast differentiation factors. It was further confirmed that OPGL is identical to TRANCE/RANKL, which was earlier found to promote T cell growth and dendritic cell function. Amino acid sequence analysis further showed that OPGL is the same molecule as tumor necrosis factor-associated activation-inducing cytokine (TRANCE) and nuclear factor κB receptor activator ligand (RANKL).

The TNFSF11 (RANKL) gene is located on human chromosome 13q14 and contains nine exons encoding nuclear factor κappaβ (NF-κB) receptor activating factor ligand (RANKL), and the encoded product of the RANKL gene is an important cytokine in the OPG-RANKL-RANK system [18]. TNFSF11 is a transmembrane protein secreted by osteoblasts and active T cells that promotes OC function and is the most important factor in inducing OC maturation [19]. TNFSF11 binds to the nuclear factor-κB receptor activation factor (receptor activator of nuclear factor-κB, RANK) to promote the differentiation, activation, survival, and attachment of OCs to the bone surface, thus exerting the function of bone resorption [20]. In our study, the expression of TNFSF11 in both adults and children was higher in cholesteatoma than in the external auditory canal skin, which indicated that higher expression of TNFSF11 was an important cause of bone destruction in cholesteatoma. Similar to the findings of Jeong et al. [21] and Chen, Qin and Lu [22], it was confirmed that compared with adult cholesteatoma, higher expression of TNFSF11 led to more OC formation and caused more severe bone absorption in pediatric cholesteatomas. The present study also found that the expression of TNFSF11 in cholesteatoma was significantly higher in children than in adults, suggesting that more TNFSF11 was activated in children with cholesteatoma, which is consistent with the results of Chen, Qin and Lu [22].

TNFRSF11B protein is a new member of the tumor necrosis factor receptor superfamily newly discovered in 1997 and is known as osteoclastogenesis inhibitory factor (OCIF or osteoprotegerin, OPG), which is an inhibitor of bone destruction and resorption. OCIF or osteoprotegerin (OPG) is a secreted glycoprotein that can inhibit bone destruction and resorption, inhibit osteoclast differentiation and maturation, and participate in the regulation of bone density. Existing studies have shown that Tnfrsf11b protein is a core member of the RANK-RANKL-OPG system, which plays a central role in the pathogenesis of osteoporosis, rheumatoid arthritis, bone cancer, and other bone dysregulation diseases. Specifically, RANKL expressed by osteoblasts and bone marrow stromal cells promotes osteoclast differentiation and bone resorption activity when it binds to RANK on the surface of osteoclasts or osteoclast precursor cells. The OPG/RANK/RANKL system is involved in the basic process of bone destruction. The human TNFRSF11B gene is located on chromosome 8q24, is 28,587 bp in length, and consists of five exons and five introns. TNFRSF11B secreted by osteoblasts in bone tissue has the effect of clearing membrane-bound soluble TNFSF11 and preventing the binding of TNFSF11 and RANK, thus inhibiting the maturation and differentiation of OCs, resisting bone resorption, increasing bone mass, and producing a bone-protective effect [23–25].

In our study, no significant difference was noted in the expression of TNFRSF11B between children and adults with cholesteatoma, indicating that TNFRSF11B has an equivalent osteoprotective effect in patients of different ages with middle ear cholesteatoma compared with the unremarkable effect of TNFRSF11B in children and adults, as suggested by Chen, Qin and Lu [22]. However, Li et al. [26] found that multiple signal couplings between TNFRSF11B and the development and maturation of OCs may be related to age, suggesting differences in the bone-protective effect of TNFRSF11B in patients with middle ear cholesteatoma.

These findings indicate that the TNFSF11-RANK-TNFRSF11B system is the key link that regulates OC function [27]. TNFRSF11B and TNFSF11 competitively bind to RANK, and the relative amounts of TNFSF11 and TNFRSF11B in the bone tissue microenvironment (i.e., TNFSF11/TNFRSF11B value) determine whether OCs are activated or inhibited [28, 29]. A high TNFSF11/TNFRSF11B ratio is the main molecular mechanism that activates OCs and plays a role in the bone resorption function [30]. The present study confirmed that higher expression of TNFSF11 was detected more often in children with middle ear cholesteatoma than in adults, and bone destruction was more severe,

while no obvious difference was noted in the expression of TNFRSF11B among patients of different age groups, and no difference was noted in the bone-protective effect. Thus, the TNFSF11/TNFRSF11B ratio was higher in children than in adults, which is an important pathophysiological factor in children with middle ear cholesteatoma being more destructive and invasive than that in adults.

Further, our study found no significant correlation between TNFSF11 and TNFRSF11B in middle ear cholesteatoma in both children and adults, and the degree of ear bone destruction was positively correlated with TNFSF11 but not associated with TNFRSF11B. The results of these correlation analyses are consistent with the results of bone destruction and TNFSF11 and TNFRSF11B content comparison between the two age groups in our study.

This study has some limitations. The majority of current studies have relatively restricted sample sizes and might not be completely representative of all patients with middle ear cholesteatoma. The pathogenesis and cytokine expression of middle ear cholesteatoma may vary among different regions and ethnic groups, and existing studies might not fully encompass these diversities. The study involved semi-quantitative detection of cholesteatomas of 50 patients at the histological level, without examining the mechanism by which TNFSF11 and TNFRSF11B affect cholesteatoma. Although it can offer certain information, there exist certain subjectivity and errors. The quantitative analysis of cytokine expression is not precise enough to accurately assess its subtle changes at the cellular and molecular levels as well as its dynamic relationship with bone destruction. In the future, more data of patients with cholesteatoma should be analyzed quantitatively, and the mechanism of occurrence and development of cholesteatoma should be further studied at the cellular or genetic level.

This study is an important supplement to the current understanding of the pathogenesis of cholesteatoma and explains the reason why cholesteatoma in the middle ear of children is more serious than that in adults. The findings of this study are novel, and the expected research purpose was achieved; these findings have guiding significance for the clinical treatment of cholesteatoma, especially in children. Future studies should revolve around the mechanism of TNFSF11 and TNFRSF11B in middle ear cholesteatoma, differences between children and adults, targeted therapy, interactions with other factors, and long-term follow-up, so as to provide a more in-depth theoretical basis and effective treatment strategies for the diagnosis and treatment of middle ear cholesteatoma.

5. Conclusions

In conclusion, the expression of TNFSF11 in cholesteatoma was significantly higher in children than in adults, which leads to more serious destruction of the ear bone in pediatric patients. This is one of the important molecular biological mechanisms underlying the destructive and invasive nature of pediatric middle ear cholesteatomas. Reducing the expression of TNFSF11 is an effective way to treat middle ear cholesteatoma. The study findings can help deepen our understanding of the mechanism of bone destruction in cholesteatoma, which has important significance in the treatment of middle ear cholesteatoma, and thus help in developing new ideas for the clinical treatment of cholesteatoma.

Author Contributions

Writing and Literature Review, S.T.; Data Collection and processing, M.Y.; Analysis and Interpretion, Z.L.; Materials, J.Y.; Design and Supervison, Z.Z.; Conception and Critical Review, Q.L. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Zhuhai People's Hospital.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement

The authors declare that the data can be shared and is publicly available. All data generated or analysed during this study are included in this published article.

Conflicts of Interest

The authors declare no conflict of interest.

Appendix A

Table A1. Details of children and adults with middle ear cholesteatoma.

Group	Example Number	Diploid Mastoid (Cases)	Sclerotic Mastoid (Cases)	Disease Course (Years)
Children group	18	14	4	4.7±3.1
Adult group	32	25	7	5.1±3.4

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