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Expression of periostin in the epithelium of cholesteatoma with different degrees of ossicular chain destruction and its clinical value in predicting postoperative hearing recovery

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Abstract

Objectives: To explore the expression of periostin in the epithelium of cholesteatoma with different destruction degrees of the ossicular chain and its clinical value in predicting postoperative hearing recovery.

Methods: Retrospective analysis was conducted on the clinical data of 100 patients with middle ear cholesteatoma (the cholesteatoma group) admitted to our hospital. Another 100 patients without middle ear cholesteatoma treated in our hospital during the same period were included in the non-cholesteatoma group. Middle ear cholesteatoma patients were further divided into a normal group, a partial destruction group, and a complete destruction group based on the destruction degree of the ossicular chain (Maresh grading). After the treatment, 75 cases were considered as the effective group and 25 cases as the ineffective group. The expression of tumor necrosis factor-alpha, Interleukin 6, and periostin in the epithelium of middle ear cholesteatoma patients with different destruction degrees of the ossicular chain and different therapeutic effects were compared. The correlation between periostin and inflammatory factors was analyzed using Pearson analysis. The predictive value of tumor necrosis factor-alpha, Interleukin 6, and periostin on treatment effect was valued using the receiver operating characteristic curve.

Results: Patients in the cholesteatoma group had a much higher content of tumor necrosis factor-alpha, Interleukin 6, and periostin than those in the non-cholesteatoma group ($P < 0.001$). The expression of tumor necrosis factor-alpha, Interleukin 6, and periostin was also largely increased with the destruction group of the ossicular chain. Patients in the ineffective group had much higher expression of tumor necrosis factor-alpha, Interleukin 6, and periostin than those in the effective group ($P < 0.001$). The Pearson correlation analysis results showed that periostin was positively correlated with the content of tumor necrosis factor-alpha and interleukin 6 ($P = 0.868$, 0.880, $P < 0.001$). The areas under the curve of individual or joint tumor necrosis factor-alpha, Interleukin 6, and periostin were 0.627, 0.793, 0.822, and 0.892, respectively.

Conclusions: The expressions of periostin, Interleukin 6, and tumor necrosis factor-alpha were markedly increased in the epithelium of middle ear cholesteatoma patients, which were gradually increased with the aggravation of the ossicular chain destruction. Periostin, Interleukin 6, and tumor necrosis factor-alpha could be used as important indicators to predict postoperative hearing recovery.

Keywords: Periostin, Destruction degrees of ossicular chain, Cholesteatoma, Postoperative hearing recovery

Introduction

Middle ear cholesteatoma (MEC) is a destructive, locally invasive lesion of the middle ear induced by inflammation, which usually occurs in the middle ear, upper tympanic cavity, mastoid process, and petrous apex. Its pathological feature is excessive proliferation and accumulation of keratin, leading to local bone destruction and inflammatory response [1]. MEC can be divided into two categories: congenital and acquired cholesteatoma. Congenital cholesteatoma accounts for about 2–5% of cholesteatoma and is mostly unilateral. Acquired cholesteatoma is often associated with chronic suppurative otitis media [2]. Surgical resection is currently the main treatment method for MEC, which aims to completely remove the lesion, protect important structures, and possibly restore hearing with artificial ossicle implantation. However, postoperative recurrence is relatively high, which can reach up to 40% after 10 years, seriously affecting the patient's hearing and quality of life [3]. At present, the pathogenesis of MEC is not fully understood, and although research hotspots focus on the characteristics of cholesteatoma epithelium, cytokines, and bioactive molecules, there is no consensus [4]. In addition, although high-resolution CT of the temporal bone is commonly used in clinical practice to assist in analyzing the extent of middle ear lesions and the integrity of the ossicular chain, it is often difficult to determine the condition of the ossicular chain (such as partial or complete destruction), and often needs to be confirmed during surgery [5]. The ossicular chain plays a vital role in sound conduction. Once destroyed, varying degrees of hearing damage may occur due to impaired middle ear transmission function [6]. A comprehensive understanding of the destroyed level of the ossicular chain is crucial for reconstructing the ossicular chain and restoring hearing.

With the advancement of imaging technology, CT and other imaging scans have high value in diagnosing whether the bone is damaged, but there are still certain limitations in diagnosing the degree of ossicular chain damage. Periostin belongs to one of the members of the bundle-forming protein family, with a molecular weight of approximately 90 kD, and is widely present in various tissues, organs, and body fluids of the human body. Studies have found that periostin is involved in the onset and progression of various diseases such as osteoarthritis [7], asthma [8], rhinitis [9], and lung tumors [10]. The level of periostin may be closely related to poor prognosis in patients. As has been reported, the periosteal protein binds to integrin to support the adhesion and migration of epithelial cells. Periostin also enhances the binding of BMP1 to the connection matrix of connective tissue to promote the structural integrity of connective tissue [11]. Meanwhile, periosteum proteins are mainly induced by type 2 cytokines IL-4 and IL-13 and are highly expressed in patients with allergic

diseases [12]. However, the relationship between periostin and the degree of ossicular chain damage is not yet clear. Whether periostin can predict postoperative hearing recovery in patients remains to be explored.

In this study, 100 MEC patients treated in our hospital from January 2019 to May 2023 were picked as the research subjects and grouped based on the degree of ossicular chain destruction and treatment effectiveness. We investigated the expression of periostin in the epithelium of cholesteatoma with different destruction degrees of the ossicular chain and related clinical value in predicting postoperative hearing recovery, aiming to provide some reference for the diagnosis and treatment of MEC in the clinic.

Results

Comparison of general data between groups

There existed no significant difference in age, gender, course of otopyorrhea, air conductive hearing in the ear,and surgical methods between the non-cholesteatoma group and the cholesteatoma group ($P > 0.05$, Table 1).

Expression of related factors in patients with different hearing impairments

There were significant differences in multiple biological indicators in the cholesteatoma group compared with the non-cholesteatoma group. Specifically, the levels of TNF-α, IL-6, and periosteum protein in the cholesteatoma group were significantly increased, and the differences were statistically significant ($P < 0.001$). These differences not only revealed the uniqueness of inflammation and extracellular matrix changes in patients with cholesteatoma, but also provided new clues and ideas for further exploring the pathogenesis and therapeutic targets of cholesteatoma (Table 2).

Table 1 Comparison of general data between groups ($\bar{x} \pm s$,%)

General data	Non cholesteatoma group ($n = 100$)	Cholesteatoma group ($n = 100$)	t/χ^2	P
Age (year)	38.22 ± 7.95	37.82 ± 5.87		
Gender				
Male	68 (68.00)	62 (62.00)	0.791	0.374
Female	32 (32.00)	38 (38.00)		
The course of otopyorrhea (year)	14.86 ± 4.85	15.32 ± 4.27		
Air conductive hearing in ear				
<55 dB	39 (39.00)	28 (28.00)	4.910	0.086
56–75 dB	35 (35.00)	32 (32.00)		
>75 dB	26 (26.00)	40 (40.00)		
Surgical method				
Open mastoidectomy	29 (29.00)	37 (37.00)	3.277	0.194
Complete bridge mastoidectomy	30 (30.00)	34 (34.00)		
Upper tympanic cavity incision and reconstruction surgery	41 (41.00)	29 (29.00)		

Table 2 Expression of related factors in patients with different hearing impairments ($\bar{x} \pm s$)

Groups	Cases	TNF-α (ng/L)	IL-6 (ng/L)	Periostin
Cholesteatoma group	100	19.55 ± 6.47	0.74 ± 0.25	1.02 ± 0.12
Cholesteatoma group	100	47.30 ± 9.52	3.41 ± 0.72	4.31 ± 0.84
t		24.109	35.032	38.773
P		< 0.001	< 0.001	< 0.001

Expression of related factors in the epithelium of cholesteatoma with different destruction degrees of ossicular chain

The expression of TNF-α, IL-6, and periostin were different among the normal group, the partial destruction group, and the complete destruction group (F = 129.06, 145.43, 101.89), $P < 0.001$). The partial destruction group had much higher expression of TNF-α, IL-6, and periostin than the normal group ($P < 0.001$). The complete destruction group had markedly higher expression of TNF-α, IL-6, and periostin than the partial destruction group ($P < 0.001$, Table 3).

Expression of related factors in patients with different treatment effectiveness

The biological indicators in the normal group showed an abnormal trend of change compared with the effective group. Specifically, the levels of TNF-α, IL-6, and periosteal protein in the ineffective group were significantly increased compared with the effective group ($P < 0.001$). This finding suggested that there might be a more intense inflammatory response in the ineffective group of patients, which might be closely related to treatment failure and disease progression (Table 4).

Correlation between Periostin and inflammatory factors

Pearson correlation analysis revealed the internal relationship between periosteum protein and TNF-α and IL-6. The results showed that the expression level of periosteum protein was positively correlated with the levels of TNF-α and IL-6 ($P = 0.868$, 0.880,

Table 3 Expressions of related factors in the epithelium of cholesteatoma with different destruction degrees of ossicular chain ($\bar{x} \pm s$)

Groups	Cases	TNF-α (ng/L)	IL-6 (ng/L)	Periostin
The normal group	22	11.48 ± 3.35	0.55 ± 0.12	1.78 ± 0.27
The partial destruction group	44	36.39 ± 10.51***	2.03 ± 0.67***	3.62 ± 1.08***
The complete destruction group	34	50.27 ± 8.85***###	3.94 ± 1.02***###	5.57 ± 1.13***###
F		129.06	145.43	101.89
P		< 0.001	< 0.001	< 0.001

*** $P < 0.001$ compared with the normal group, ### $P < 0.001$ compared with the partial destruction group

Table 4 Expressions of related factors in patients with different treatment effectiveness ($\bar{x} \pm s$)

Groups	Cases	TNF-α (ng/L)	IL-6 (ng/L)	Periostin
The effective group	75	12.33 ± 2.54	0.53 ± 0.14	1.39 ± 0.33
The ineffective group	25	56.75 ± 16.59	3.66 ± 1.26	5.71 ± 1.25
t		22.625	21.334	27.436
P		< 0.001	< 0.001	< 0.001

Table 5 Correlation between Periostin and inflammatory factors

	Periostin	
	r	*P*
TNF-α	0.868	0.880
IL-6	< 0.001	< 0.001

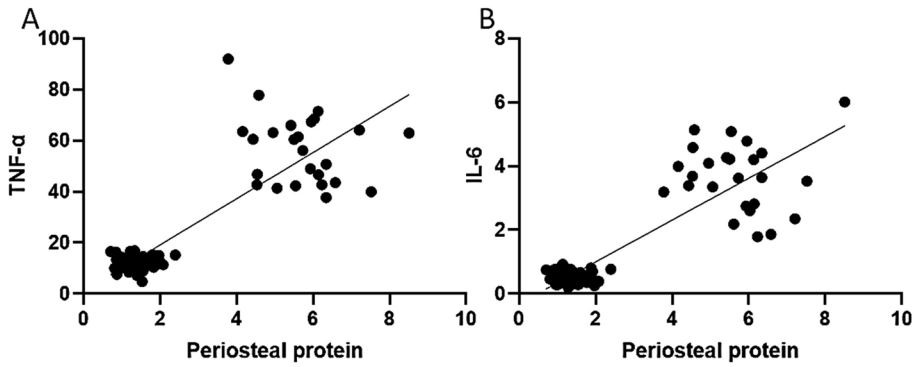

Fig. 1 Correlation between Periostin and inflammatory factors. **A** Correlation between Periostin and TNF-α; **B** Correlation between Periostin and IL-6

Table 6 Predictive value of TNF-α, IL-6, and periostin for therapeutic efficacy

Indicators	AUC	Sensitivity	Specificity	Cutoff value	Jordan index	*P* value	95% *CI*
TNF-α	0.627	56.00%	89.33	27.36 ng/L	0.453	0.042	0.458–0.797
IL-6	0.793	64.00%	82.67	1.37 ng/L	0.467	0.004	0.697–0.889
Periostin	0.822	96.00	60.00	3.65	0.560	0.001	0.724–0.920
Combined detection	0.892	95.20	81.20	–	0.760	0.001	0.830–0.953

$P < 0.001$). These data suggested that when the levels of TNF-α and IL-6 were increased, the levels of periosteal protein were also increased (Table 5; Fig. 1).

The predictive value of TNF-α, IL-6, and periostin for therapeutic efficacy

Establish ROC curve was established. The AUC of individual and combined detection was 0.627, 0.793, 0.822 and 0.892, respectively. Combined detection had a higher predictive value for the treatment effect of MEC patients ($P < 0.05$, Table 6; Fig. 2).

Discussion

MEC is one of the ear, nose, and throat diseases, characterized by abnormal proliferation of keratinized squamous epithelium in the temporal bone, and can gradually destroy surrounding bone. Research has found that MEC may induce intracranial and extracranial complications, including hearing loss, dizziness, facial paralysis, etc. [13, 14]. MEC is a benign lesion with a proliferation, invasion, and migration pattern similar to malignant tumors. Imaging examination also reveals bone destruction and increased density in the middle ear cavity [15, 16]. Under normal circumstances, sound waves are transmitted to the eardrum through the auditory bone chain, causing

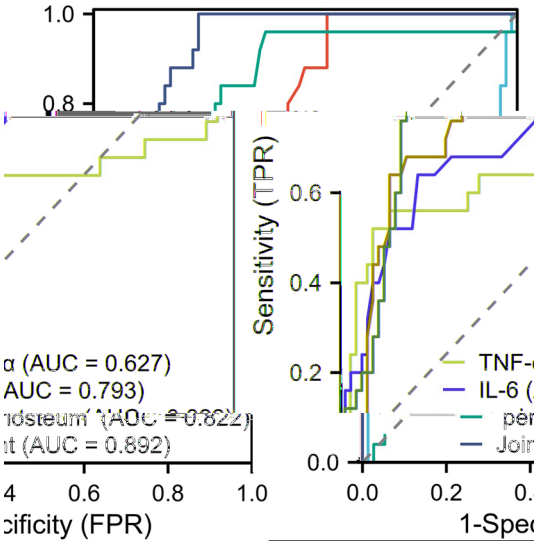

Fig. 2 ROC curve analysis of the predictive value of Periostin for therapeutic efficacy

lymph fluid vibration and transmitting sound. However, preliminary research has found that the probability of hearing recovery is higher due to the disruption of the ossicular chain [17]. Surgery is the main method for treating MEC, but the postoperative recurrence rate is relatively high [18]. Exploring the pathogenesis of MEC can reveal indicators related to the degree of ossicular chain destruction, providing new ideas for predicting hearing recovery and preventing and treating MEC.

Periostin is an extracellular matrix-secreted protein that can be expressed in the dermis of the skin. Research has found that the expression of periostin is significantly increased when skin damage is caused by diseases such as dermatitis and autoimmune reactions [19, 20]. Periostin may participate in the onset and progression of diseases as an inflammatory factor. The skin in cholesteatoma differs histologically from normal skin, exhibiting a squamous keratin epithelial matrix separated by an inflammatory layer rich in lymphocytes and mast cells. Lymphocytes and mast cells are considered to play important roles in clinical symptoms and complications [21]. This study found that the expression of periostin in the epithelial tissue of MEC was significantly increased, and it also gradually increased with the severity of ossicular chain destruction. Therefore, we inferred that periosteal proteins might be involved in the development of MEC. Analyze the reasons behind it, the damage of MEC to surrounding tissues occurs in the chronic inflammatory micro-environment. The epithelium of cholesteatoma may exhibit a proliferative state in the inflammatory microenvironment, while the expression of periostin is significantly increased during the inflammatory response, further exacerbating the progression of the disease [22].

Cytokines are a type of low molecular weight proteins used for intercellular communication, which can be secreted under various stimuli and interact with their receptors to jointly regulate cell function [23]. TNF-α is a cytokine closely related to inflammation and immune processes, which can be secreted by lymphocytes and monocytes. A study has found that TNF-α can directly act on the differentiation and

maturation of osteoclasts, and indirectly expose the bone matrix, thereby affecting bone destruction and remodeling [24]. Inflammatory mediators may play an important role in the pathogenesis of otitis media by initiating and maintaining an inflammatory response to infection. IL-6, as an inflammatory factor, can induce the production of C-reactive protein (CRP) and participate in regulating cell proliferation and differentiation in anti-infection responses [25]. A study has found that serum IL-6 levels in patients with otitis media are significantly elevated, which can induce the formation of osteoclasts and play a certain role in the development of otitis media [26]. The results of this study found that the level of IL-6 was significantly increased in MEC patients, which also increased with the degree of ossicular chain destruction. Therefore, we inferred that IL-6 might be related to the degree of ossicular chain destruction and the severity of the condition. In addition, the Pearson correlation analysis showed that the expression of periostin was positively correlated with the content of TNF-α and IL-6. The AUC of individual and combined detection was 0.627, 0.793, 0.822 and 0.892, respectively. Combined detection had higher predictive value for the treatment effect of MEC patients. The above results also indicated that the expression of TNF-α, IL-6, and periostin might have certain predictive value for postoperative hearing recovery. Therefore, in clinical practice, the expression level of periostin can be detected to predict postoperative hearing recovery, providing a more targeted basis for clinical treatment.

In general, the expression of periostin, IL-6, and TNF-α was markedly increased in the epithelium of MEC patients, which was gradually increased with the aggravation of the ossicular chain destruction. Periostin, IL-6, and TNF-α could be used as important indicators to predict postoperative hearing recovery. However, this study still had certain limitations, as only adults were included in the study due to time and funding constraints. It is not yet known whether children have the same results. In the future, the sample size can be expanded and children will be included as research subjects to further verify the results.

Materials and methods

General materials

The retrospective analysis proceeded with the clinical data of 100 MEC patients (defined as the cholesteatoma group) admitted to our hospital from January 2019 to May 2023. The cholesteatoma group was made up of 62 males and 38 females aged 21–65 years, with an average age of (37.82 ± 5.87). Inclusion criteria: (1) all patients met the diagnostic criteria for MEC [27]; (2) all patients aged between 18 and 60 years; (3) all patients had complete clinical data; and (4) patients accompanied by clinical manifestations such as long-term ear discharge, bloody streaks, and special foul odor. Exclusion criteria: (1) those with concomitant neurological deafness; (2) patients with combined middle ear infections; (3) patients with congenital developmental dysplasia or malformation of the middle ear; and (4) patients treated with antibiotics or other medications before participating in the study. Another 100 patients without MEC treated in our hospital during the same period were included as the non-cholesteatoma group [68 males and 32 females aged 20–65 years, with an average age of (38.22 ± 7.95)]. The two groups had no significant difference in general materials such as age and gender ($P > 0.05$).

MEC patients were further divided into a normal group (22 cases with grade I), a partial destruction group (44 cases with grade II + grade III), and a complete destruction group (34 cases with grade IV) based on the destruction degree of ossicular chain (Maresh grading) [28]. All patients underwent surgical treatment, including tympanoplasty, mastoidectomy, modified mastoidectomy, mastoidectomy, upper tympanoplasty, bridged mastoidectomy, external ear canal shaping, and turbinate cavity shaping, as well as related or complementary surgeries for these surgeries. Based on the treatment effect of the patient after 6 months of treatment, 75 cases were considered as the effective group (ear pain and otopyorrhea were completely disappeared or improved, and the average hearing loss frequency increased by 15 dB or more), and 25 cases as the ineffective group (no significant improvement or even aggravation of clinical symptoms or hearing and the average increase in hearing loss frequency was less than 15 dB).

Specimen collection

Specimen collection was performed by an experienced chief otologist who collected MEC epithelium at the entrance of the tympanic sinus during surgery. Diseased mucosal tissue was collected from non-cholesteatoma patients. After rinsing the blood with sterile physiological saline, the specimen was stored in a −80 °C ultra-low temperature refrigerator (Ailaibao (Medical) Medical Equipment Co., Ltd., model: BDF-86V598) for freezing.

Outcome measures

Detection of tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6)

The content of TNF-α and IL-6 in the epithelium of cholesteatoma was measured by enzyme-linked immunosorbent assay (ELISA). First, serum samples were taken from the patient. Venous blood samples were drawn in the early morning on an empty stomach, and then centrifuged to separate the upper serum, and stored at −80 °C to be measured. The specific anti-TNF-α antibody or anti-IL-6 antibody was pre-coated on a 96-well microplate to form a solid phase carrier. Then, the standard, positive control, and test samples were added to the corresponding Wells and incubated at room temperature for 2 h or overnight at 4 °C. After washing, biotin-labeled TNF-α or IL-6 was added to detect antibodies and incubated again for 1 h. Subsequently, horseradish peroxidase (HRP) labeled Streptavidin–HRP was added and incubated for 45 min. After washing again, the TMB substrate solution (Tianen Biochemical Technology (Beijing), PA107) was added and incubated for 30 min under dark conditions. The absorbance (OD value) was immediately determined at 450 nm by adding the termination solution. OD values for each well were obtained using a Microplate Reader (Thermo Fisher Scientific, Multiskan FC), and the concentrations of TNF-α and IL-6 in the sample were calculated according to the standard curve. TNF-α Kits were purchased from Solaibao (Beijing, SEKRT-0402). IL-6 Kits were purchased from Atagenix (Wuhan, ATK00014).

Detection of periostin

Western blotting was used to detect the expression of periostin in the epithelium of cholesteatoma. The epithelium of cholesteatoma was thoroughly lysed and centrifuged at 12,000×*g* for 20 min at 4 °C. The concentration of protein was estimated using a protein

assay kit. After being separated with 8% SDS–PAGE (60 μg per lane), the proteins were electrotransferred onto polyvinylidene fluoride membranes (Shanghai Aladdin Biochemical Technology, P432377). The membrane was then incubated with periostin polyclonal antibodies (1:10,000) at 4 °C overnight. The membrane was washed 4 times in phosphate-buffered saline-Tween (PBST, Shanghai Aladdin Biochemical Technology, P196391) for 10 min each time, and then incubated with a suitable horseradish peroxidase-labeled secondary antibody (1:400) for 2 h. GAPDH served as an internal control. The protein imprinting bands were imaged using an enzyme chemiluminescence (ECL) assay kit (Betterarray, Wuhan, CCT-QBD-60012-200) and quantitatively analyzed using ImageJ software. All experiments should be repeated at least three times.

Statistical analysis

In this study, measurement data such as TNF-α, IL-6, and periostin were tested for normal distribution and all conformed to normal distribution. The measurement data were all expressed in the form of ($\overline{x} \pm s$). An Independent sample t test was used for the measurement data between two groups. Multiple group comparisons were conducted using a one-way analysis of variance, and further pairwise comparisons were conducted using the LSD-t method for testing. Receiver operating characteristic (ROC) curve analysis was established for predicting value analysis; Pearson analysis was conducted for the correlation between periostin and inflammatory factors. In this study, SPSS 22.0 software was used for statistical data analysis, and $P < 0.05$ was considered as the difference with statistical significance.

Author contributions

Cuncun Xie, Guangke Wang and Hongjian Liu confirmed the authenticity of all the raw data and edited the manuscript, Xiaodong Jia and Shaoguang Ding collected data and processed the data. Cuncun Xie and Xiaoli Ding conducted the statistics. Guangke Wang and Hongjian Liu reviewed and revised the article. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by The Ethics Committee of Henan Provincial People's Hospital (20230702). Informed consent was obtained from participants for the participation in the study and all methods were carried out in accordance with relevant guidelines and regulations.

Competing interests

The authors declare no competing interests.

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