Indian Journal of Biochemistry & Biophysics
Vol. 60, August 2023, pp. 624-631
DOI: 10.56042/ijbb.v60i8.4299

Upregulation of Psoriasinin Cholesteatoma associated with inflammation, bone destruction and severity of disease: Histo-cyto-chemical and ultrastructural studies

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Received 11 April 2023; *revised 20 July 2023*

Cholesteatoma remains a mystery and the factors triggering and propagating it unknown. This study was aimed to evaluate the role of Psoriasin and pro-inflammatory cytokines in progression of middle ear cholesteatoma. In this study 18 patients (12 cases of cholesteatoma and six cases of posterosuperior retraction pocket (PSRP) without cholesteatoma) were included in the study. Clinical aggressiveness was evaluated on-table under operative microscope by evaluating erosion of ossicles, invasion of surrounding structures. The classification of disease was also based on the radiological (CT-scan) findings. Based on clinical aggressiveness, the cholesteatoma cases were divided into mild, moderate, and severe. The tissues were processed for histopathological, ultrastructural and immunohistochemical analysis. The Psoriasin expression in cholesteatoma tissues was significantly higher ($P< 0.05$) compared to the PSRP tissue and increased with the increasing severity of the disease. The pro-inflammatory cytokines (IL-6 and IL-1β) were also upregulated along with vascular endothelial growth factor and Matrix metalloproteinase-13 in cholesteatoma cases compared to control. Transmission Electron Microscopic images of ultra-thin sections of the tissues showed numerous secretory vesicles, most likely containing cytokines and a unique ultrastructural feature of bacterial localization within the membrane pocket was seen, which gave direct evidence of bacterial infection in the middle ear cholesteatoma.

Keywords: Bacterial localization, Cholesteatoma, Histo-cyto-chemistry, Psoriasin, Tympanomastoidectomy, Ultrastructure

Cholesteatoma of the middle ear develops due to abnormal epithelial migration resulting in accumulation of keratin debris of the squamous epithelia. The disease is characterized by rapid destruction and invasion of the surrounding tissues. The only available treatment at present is surgical intervention. The cholesteatoma patients may suffer from severe, possibly fatal complications including meningitis and brain abscess. The exact aetiology, molecular mechanism of disease development, and progression is largely unknown[1]. Understanding of the molecular mechanisms involved in the pathogenesis and development of cholesteatoma is important for the development of new medical treatment options. The induction of abnormal epithelial migration in the middle ear is considered to be arising from negative pressure resulting in retraction-pockets formation. The other considerations for the cholesteatoma development include keratinocytes migration from external auditory-canal (EAC) through an already existing perforation, middle ear mucosal metaplasia remnants of embryonic cells[2]. keratinocytes in the tympanic cavity does not essentially result in development of cholesteatoma The hypothesis is strengthened by many reports showing relatively low incidence of cholesteatoma formations as a result of perforations in tympanic membrane[3]. The hypothesis is also supported by the findings of non-uniformity in success rates of animal skin graft in cholesteatoma experimental models. Therefore, it is thought that various unknown causative factors may be involved in the middle ear cholesteatoma formation. The role of various molecular and immunological factors, including infection[5], inflammation[6], angiogenesis[7], proliferation[8], apoptosis[9], bone erosion and altered lipid metabolism[10] have been investigated in cholesteatoma formation and progression[11]. These studies have shown inflammatory changes and bone erosion, while the direct evidence of the presence of bacterial

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pockets at the site of cholesteatoma formation is not yet available.

During the development of cholesteatoma, various antimicrobial peptides are produced, including cathelicidin[12] and defensins[13,14], which are considered to be 'chemical barriers' for the infection. The variants of calcium binding protein homologous to S100 have also been identified. These proteins play a role in calcium-dependent signalling pathways. Psoriasin (S100A7) is a protein belonging to the S100 gene family. The Psoriasin expression has been identified in the cells of squamous epithelium isolated from the skin lesion of psoriasis patients, while it was not expressed in normal skin epithelium[14]. The Psoriasin is an antimicrobial peptide and oxidative stress[15], and a chemotactic agent, facilitating recruitment of neutrophils and lymphocytes at the site of its expression[16]. In the presence of calcium and retinoic acid, the expression of Psoriasin in keratinocytes gets up-regulated[17]. An increased expression of Psoriasin in cholesteatoma has been reported recently[18]. This finding prompted us to evaluate Psoriasin expression in relation to disease progression. The present study was performed to investigate the ultrastructural and histopathological features of cholesteatoma along with the evaluation of the role of Psoriasin, IL-6, IL-1b, MMP-9 and VEGF in the severity of middle ear cholesteatoma.

Material and Methods

This study was conducted from January 2016 to January 2018, with the collaboration of the Department of ENT and Dept of Anatomy, at our tertiary care referral centre, after getting approval from the Institute Ethics Committee (IEC). Eighteen patients (12 cases of cholesteatoma and six cases of posterosuperior retraction pocket {PSRP}) were included in the study after obtaining written consent from the patients and/or relatives of the patients. The cholesteatoma cases were taken as study group and PSRP cases were considered as control group. The diagnosis was made by otoscopic examination, and a high-resolution computed tomography (HRCT) scan of the temporal bone was obtained for all the cholesteatoma patients to grade the severity of the disease.

Tissues Collection

The cholesteatoma matrix was obtained intra operatively from the patients undergoing tympanomastoidectomy.

Tissue preparation

Immediately after surgery desired tissues were cut into two parts. One part was immediately dipped and fixed in 4% paraformaldehyde (0.1 M phosphate-buffered) saline solution (PBS, pH 7.4) for 24 h at 4°C. The tissue was then embedded in paraffin for histopathological examinations and subjected to cryo-sectioning for Immunofluorescence and Immunohistochemistry. The other portion of the tissue was fixed in Karnovsky solution and processed for ultrastructural analysis using transmission electron microscopy. The tissues were processed for ultrastructural examinations as follows:

Fixation: The tissue was fixed in 2.5% glutaraldehyde and 2% paraformaldehyde, for 12–24 h at 4°C, followed by washing 4–5 times in 0.1 Molar PBS.

Post-fixation: The cholesteatoma tissue was again fixed in 1% osmium tetroxide for 2 h at 4°C and washed 4–5 times (in 0.1 Molar PBS).

Dehydration: Now the fixed tissue was dehydrated by various grades of dilutions of acetone at different temperatures as previously described (Quadri *et al*., 2018).

Clearing: The tissue was dipped in toluene for 30 min for the clearing of the tissue.

Infiltration: For infiltration, LR White (acrylic resin) and toluene were used and embedded in different concentrations as follows- 1:3 (2 h at room temperature) → 2:2 (2–3 h at room temperature) → 3:1 (2 h at room temperature under vacuum condition) followed by in pure resin (2 h at room temperature) and pure resin (2 h at 50°C temperature).

Embedding and Polymerization: Finally, the infiltrated tissues were embedded in resin using BEEM capsules (Ted Pella, California, USA) and polymerized at 50°C for 12–24 h in a chamber of dry heat bath, and temperature was raised to 60°C (48–72 h) for polymerization.

Light Microscopy: The prepared blocks were cut using glass knives in 1 mM thick serial sections to locate the tissue of interest under light microscope. The sections subjected to light microscopy were stained with 1% toluidine blue (prepared in 1% borax). After finding the desired area of interest ultrathin sections (60–90 nm) were cut and mounted on 400 mesh-nickel grids.

Staining: The mounted ultra-thin sections were stained with uranyl acetate (10–15 min), followed by washing with 50% ethanol and double distilled water. After washing, the meshes were placed on drops of

lead citrate for 5–10 min and were washed again (with 0.02 Molar sodium hydroxide & double distilled water) and dried.

Viewing: The stained tissues mounted on grids were viewed under transmission electron microscope (Tecnai G2 200kV, TEM, FEI, Oregon, USA).

Immunolocalization and expressional analysis of Psoriasin, IL-1β, IL-6, VEGF, and MMP-9 proteins by Immunofluorescence (IF)and Immunohistochemistry (IHC)

Formalin-fixed and paraffin-embedded blocks were used for IHC, and cryo-sections were used for IF. For cryo-sectioning, tissues were washed thrice with PBS for five minutes each and placed in a 15% sucrose solution till it descended to the bottom of the container. The process was repeated with 30% sucrose solution and the tissues were thereafter embedded in OCT embedding matrix and blocks were prepared. Tissues were cryo-sectioned using the cryo- microtome. Gelatine coated slides were used for tissue mounting. The tissue sections were incubated in mummified chambers with anti-human antibodies (Psoriasin, IL-1β, IL-6, VEGF, and MMP-9) overnight at 4°C (Novus Biologicals, USA). Incubated samples were washed with Tris buffer (pH-7.4) and again incubated with respective fluorescent tagged secondary antibodies (Novus Biologicals, USA) in a humidified chamber for four h at 4°C. In case of IHC, colour was developed using substrate DAB (Sigma, USA).

Haematoxylin and Eosin (H&E) staining

H&E staining was performed to evaluate the histopathological changes in the cholesteatoma and PSRP tissue sections as per the standard protocol previously described.

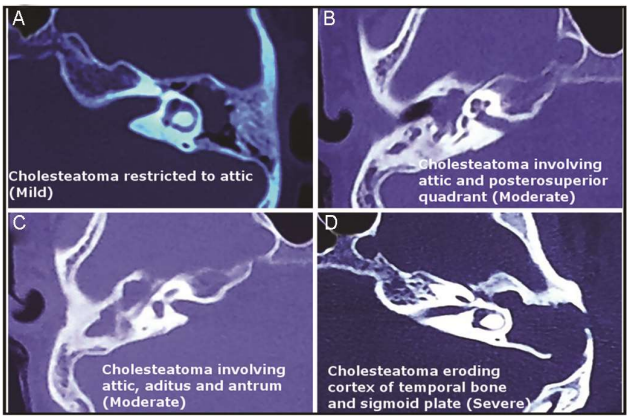

Fig. 1 — Representative CT scan images showing (A) middle ear cholesteatoma restricted to attic only (mild grade); (B) cholesteatoma involving attic and posterior-superior quadrant (moderate grade); (C) cholesteatoma involving attic, aditus and antrum; and (D) severe grade cholesteatoma showing erosion of cortex of temporal bone and sigmoid plate

Results

CT scan findings

CT scan images showed that in mild cases the cholesteatoma is restricted to epitympanum only. The cholesteatoma with moderate severity involves attic and posterior-superior quadrant. In extensively erosive severe cases the extension of cholesteatoma includes attic, aditus and antrum erosion of cortex of temporal bone and sigmoid plate (Fig. 1).

Histopathological changes in middle ear cholesteatoma

The severity of disease was determined assessing extension of disease including ossicular erosion and degrees of invasion. Histopathological changes showed strips of stratified squamous epithelium (Fig. 2). Epithelial elements were seen with keratinous contents in biopsies from cholesteatoma (Fig. 2A), while the keratinous contents were absent in PSRP (Fig. 2F). Ghost squamous cells were also observed (Fig. 3B). Inflammatory cells infiltrates (comprising of lymphocytes, histiocytes and plasma cells) were seen in abundance (Fig. 2).

Bacteria colonize and make pockets in middle ear cholesteatoma

Transmission electron microscopic (TEM) images showed strips of stratified squamous epithelium layer along with inflammatory and apoptotic cells (Fig. 3). Many secretory vesicles most likely containing cytokines were observed. The numbers of such secretory

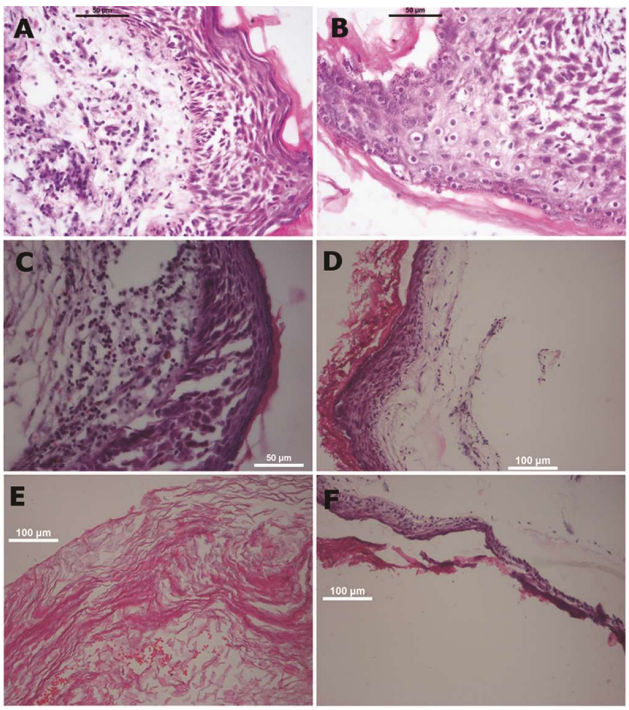

Fig. 2 — Tissue sections of cholesteatoma (A-E); and PSRP (F)

vesicles were remarkably high in severe cases compared to the mild cholesteatoma and PSRP (Fig. 3) Ultrastructural examinations revealed unique features of bacterial pockets in middle ear cholesteatoma (Fig. 4).

Psoriasin expression increases with increasing severity of cholesteatoma

Psoriasin acts as an antimicrobial peptide and a chemo-attractant for lymphocytes, neutrophils, and other immune effect or cells. We performed Immunofluorescence to evaluate the tissue levels expression of Psoriasin in cholesteatoma. A high level of Psoriasin expression was observed in severe grades of cholesteatoma compared to moderate and mild grades. The expression of Psoriasin was significantly high ($P< 0.05$) in all grades of cholesteatoma compared to PSRP (Fig. 5). The present findings indicate that intercellular communication through local mediators of inflammation; angiogenesis and microbial antigens activate overexpression of Psoriasin which leads to abnormal physiology of keratinocytes.

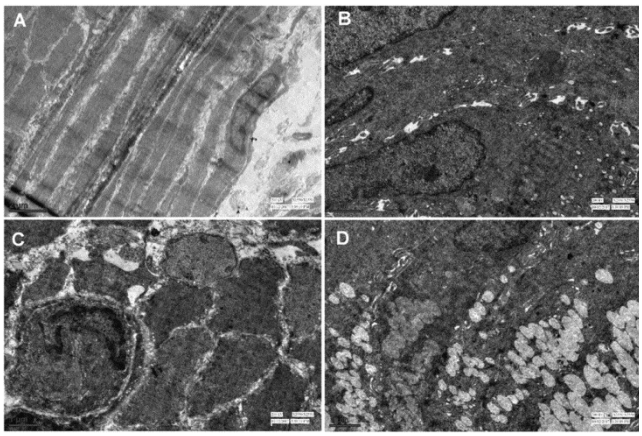

Fig. 3 — Electron micrographs of ultra-thin sections (60 nm) from middle ear cholesteatoma tissue showing (A) membrane; (B & C) inflammatory and apoptotic cells; and (D)- secretory vesicles. Scale bar a: 2 µM, b-d: 1 µM

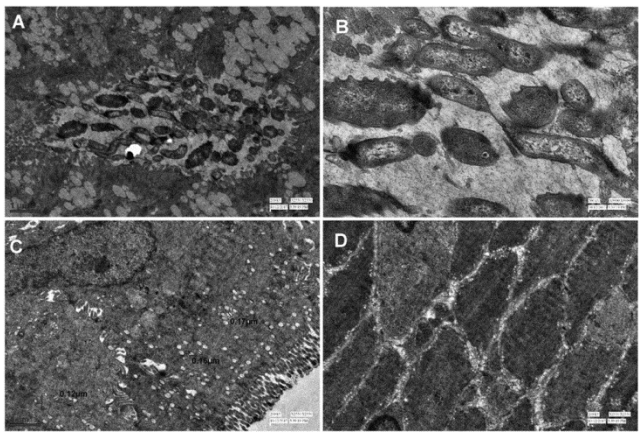

Fig. 4 — Electron micrographs of ultra-thin sections (60nm) of middle ear cholesteatoma (A-C) and PSRP epithelium (D). (A & B) are showing pockets containing bacteria, (C)-secretory vesicles along with blabbing of vesicles from apical face of membrane (D). Scale bar a, c & d: 1 µM, b: 0.2 µM (PSRP-posterosuperior retraction pocket)

IL-1β expression increases with increasing inflammation and disease extension

IL-1β is a proinflammatory cytokine and it's over expression in tissues indicates inflammatory changes. IL-1β induces production and release of acute-phase inflammatory reactants. The expression of IL-1β is also upregulated due to over production of reactive oxygen species (ROS) and oxidative damages to cells and tissues. In the present study, a significant ($P< 0.05$) increase in IL1β expression was observed in middle ear cholesteatoma (Fig. 6) compared to the controls (PSRP). The levels of expression tend to increase with increasing severity of disease.

IL-6 expression increases with increasing grades of cholesteatoma

IL-6 has been considered as a pro-inflammatory cytokine. It is the part of signalling pathways of

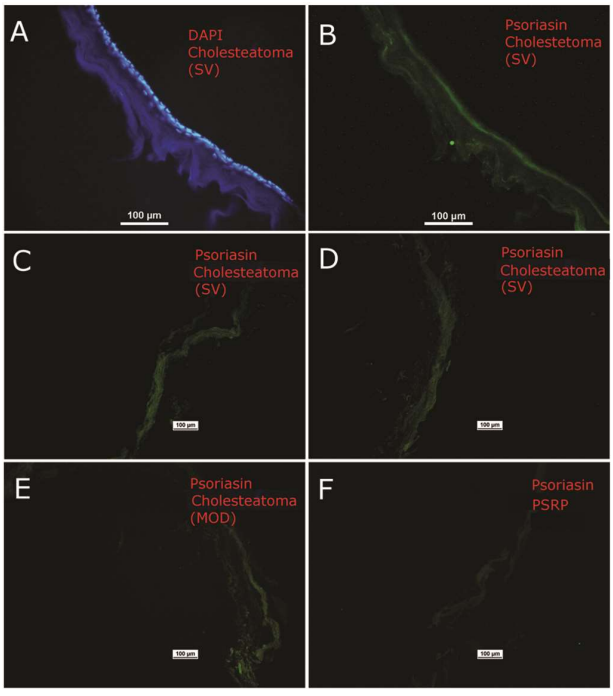

Fig. 5 — Representative immunofluorescence images of Psoriasin expression in middle ear cholesteatoma. (A) DAPI stained sections showing cellularity (blue glowing nucleus); (B-D) Psoriasin expression (green glow) in severe cases of cholesteatoma; (E) Psoriasin expression (green glow) in moderate cases of cholesteatoma; and (F) No Psoriasin expression in PSRP subjects. Scale bar 100 µM. (DAPI-4′,6-diamidino-2-phenylindole, SV- Severe disease; MOD-Moderate disease)

induction and regulation of macrophages recruitment and activation. Immunofluorescence was performed to evaluate the tissue expression levels of IL-6 in cholesteatoma in present study. Significantly ($P< 0.05$) increased level of expression was observed in severe grade of cholesteatoma as compared to mild grade of cholesteatoma (Figs 6 & 7).

Vascular endothelial growth factor (VEGF) expression increases with severity of middle ear cholesteatoma

VEGF is considered as vital for the induction of angiogenesis in the body and also induces inflammation. The expression of VEGF is influenced by local mediators including cytokines, interleukins, and growth factors. The level of expression of VEGF modulates physiological functions and blood vessels formation. The expression of VEGF may be induced by bacterial and viral antigens, toxins, cytokines, self-altered cells, hormone, and growth factors. Immunofluorescence was performed to evaluate the expression of VEGF in cholesteatoma tissue in present study. A significantly ($P< 0.05$) high level of expression was observed in severe grades of cholesteatoma compared to mild grades disease. In PSRP, VEGF expression was significantly ($P< 0.05$) lower than all grades of cholesteatoma (Figs 8 & 9). The findings showed that infection, inflammation, and epithelial cell alteration (keratinisation) in middle ear cholesteatoma is facilitated and extended rapidly due to fast angiogenesis (VEGF expression).

MMP-13 over expression aggravates bone erosion in middle ear cholesteatoma

MMP-13 has been implicated in bone erosion and osteoclastic changes. The expression and activation of MMPs are influenced by inflammation and pro- inflammatory cytokines. Immunohistochemistry was performed to evaluate the tissue expression levels of MMP-13 in cholesteatoma in present study. A high level of expression ($P< 0.05$) was observed in severe grades of cholesteatoma as compared to mild grades of cholesteatoma and PSRP (Fig. 8).

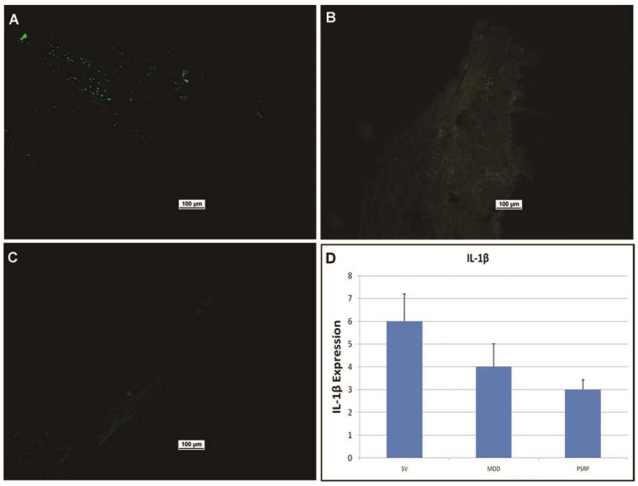

Fig. 6 — Representative immunofluorescences images of IL-1β expression (green glow) in cholesteatoma; (A) severe cases; (B) moderate cases; and (C) in PSRP subjects. (D) comparative graph of IL-1β expression. Scale bar 100 µM

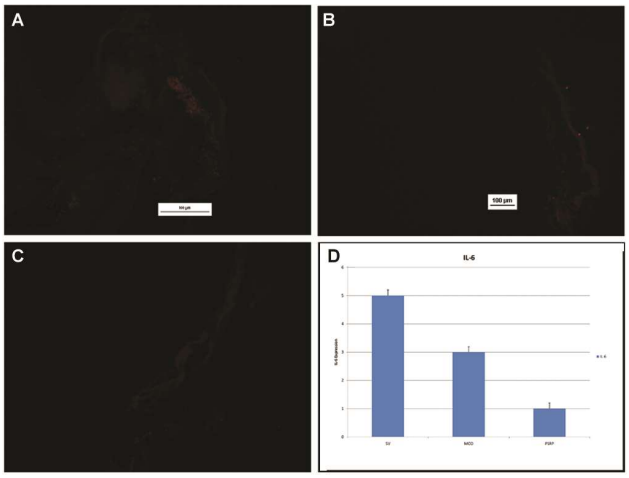

Fig. 7 — Representative immunofluorescence images of IL-6 expression (red glow) in cholesteatoma- (A) severe cases; (B) moderate cases; and (C) PSRP and (D) comparative graph of IL-6 expression. Scale bar 100 µM

Fig. 8 — representative immunofluorescence images of VEGF expression (red glow) in cholesteatoma (A) severe cases; (B) moderate cases; and (C) PSRP. Scale bar 100 µM

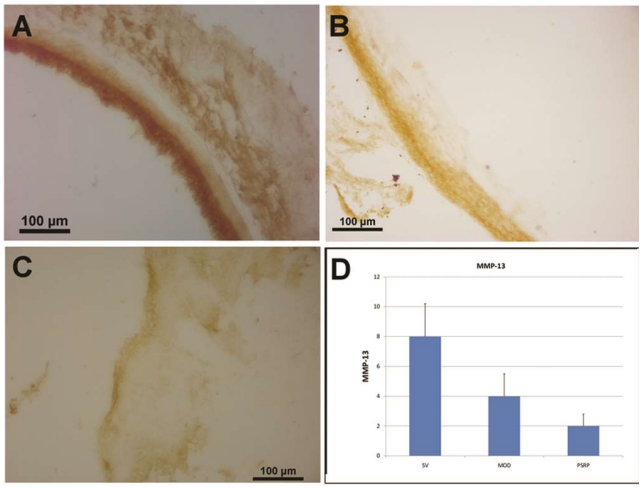

Fig. 9 — Representative immunofluorescence images of MMP-13 expression (dark brown) in cholesteatoma (A) severe cases; (B) moderate cases of Cholesteatoma; (C) PSRP; and (D) comparative graph of MMP-13 expression. Scale bar 100 µM. (MMP- Matrix metalloproteinase; PSRP- posterosuperior retraction pocket)

Discussion

The recent studies have shown that proteolytic events induced by cholesteatoma play a vital role in bone erosion. The bone erosion underlies the infiltrative nature of the disease with associated recidivism and potentially life-threatening complications. Abnormalities in the proliferation, differentiation and migration of keratinocytes are influenced by the release of inflammatory cytokines and growth factors by infiltrating immune effect or cells and activation of fibroblasts in the perimatrix[5].

Cholesteatoma induces injury to the adjoining tissues most likely due to increased production of matrix metalloproteinase (MMPs)[19]. The MMPs are calcium-zinc-dependent endopeptidases produced by various cells including endothelial cells, keratinocytes, fibroblasts and activated macrophages[20-22]. In the present study, it was observed that along with Psoriasin, IL-1β, IL-6 and MMP-13 were highly expressed in the inflamed areas (Fig. 8) while their expression was lower in PSRP cases (Fig. 8C). These findings suggest that inflammatory cytokines (Psoriasin, IL-1β and IL-6) facilitate production of MMPs.

Psoriasin is a recently identified member of the S100 gene family (S100A7). It acts as an antimicrobial peptide[23] and also plays a role in chemotaxis and stimulation of lymphocytes and neutrophils. Psoriasin is usually expressed in skin disease Psoriasis, which is an inflammatory disease.[24] The recent findings indicate that intercellular communication between innate as well as adaptive immune cells along with abnormal physiology of keratinocytes may activate inflammatory responses. In the present study, it was observed that the Psoriasis plays an important role in abnormal keratinocytes proliferation and progression of cholesteatoma. The expression of Psoriasin, IL-6 and IL-β were higher in the severe cases of cholesteatoma as compared to mild and moderate cases. The findings of this study indicate that the upregulation of VEGF expression increases blood supply to the tissues and facilitates disease progression. These findings suggest that higher expression of Psoriasin facilitates immune cell infiltration and production of various proinflammatory mediators in the middle ear cholesteatoma.

The role of bacterial infection has been suggested in the middle ear cholesteatoma, but the evidence is indirect. In the present study, the presence of bacterial pockets in the middle ear cholesteatoma was confirmed by transmission electron microscopy (TEM) (Figs 3 & 4). Antimicrobial peptides (AMP) are expressed in response to the bacterial infection and provide the first line of defence against pathogens. AMPs act as chemotactic agents for pro-angiogenic factors and immune effector cells, orchestrating inflammation, angiogenesis, and wound healing[25]. VEGF is derived from epidermis and is over expressed in many skin lesions including psoriasis. Experimental studies have shown that the upregulation of VEGF leads to hyper angiogenesis and inflammatory disorders. The Psoriasin is not a direct inducer of angiogenesis; however, it's over expression and presence in extracellular fluid influences the expression and activity of regulators of angiogenic factors[26-28].

In the present study, it was seen that the VEGF expression was highest in the cases with severe cholesteatoma. The Psoriasin expression was high in severe cases of cholesteatoma, which suggest that Psoriasin expression increases angiogenesis and leads to chronic inflammation.

Recent studies have showed that reactive oxygen species (ROS) promotes expression of Psoriasin[26]. Microbial infections induce over production of ROS as a physiological attempt to fight with the pathogens. The presences of bacterial pockets were seen in the cholesteatoma which strongly suggest induction of Psoriasin expression, which in turn induced angiogenesis and inflammation. The findings suggest

that Psoriasin blocker may be able to reduce the disease progression and recurrence.

Our study has certain limitations. We did not have a control group from the normal individuals; however, the tissue from individuals with PSRP acting as control showed that the expression of Psoriasin was significantly lower in these individuals compared to patients with full blown cholesteatoma. The small sample size limits the reliability of the statistical conclusions. The findings from a larger cohort of patients will be more assertive to strengthen these deductions. Furthermore, our findings, though implicate the role of Psoriasin in the pathophysiologic cascade of events associated with cholesteatoma, the causal relationship is at best speculative and needs further studies.

Conclusion

Our findings reveal a statistically significant rise in the expression of Psoriasin in patients with cholesteatoma compared to the patients with posterosuperior retraction pockets. The expression of Psoriasin and pro-inflammatory cytokines (IL-1β, IL-6, VEGF and MMP-13) was found to increase with increasing severity of the disease. The findings suggest the potential role of Psoriasin in the pathogenesis of disease progression in cholesteatoma. Further research is required to establish cause and effects relationship between the two.

Acknowledgement

Authors are thankful to the Electron Microscope Facility (SAIF New Delhi) for providing an opportunity to conduct ultrastructural analysis and AIIMS, New Delhi to providing financial (Intramural Research Funding) and all other supports.

Conflict of interest

All the authors declare no conflict of interest.

References

1 Pusalkar AG, Cholesteatoma and Its Management. *Indian J Otolaryngol Head Neck Surg,* 67 (2015) 201.
2 Olszewska E, Wagner M, Bernal-Sprekelsen M, Ebmeyer J & Dazert S, Etiopathogenesis of cholesteatoma. *Eur Arch Otorhinolaryngol,* 261 (2004) 6.
3 Vennix PP, Kuijpers W, Peters TA, Tonnaer EL & Ramaekers FC, Growth and differentiation of meatal skin grafts in the middle ear of the rat. *Arch Otolaryngol Head Neck Surg,* 120 (1994) 1102.
4 Jackson DG & Lim DJ, Fine morphology of the advancing front of cholesteatoma–experimental and human. *Acta Otolaryngol,* 86 (1978) 71.
5 Ricciardiello F, Cavaliere M, Mesolella M & Iengo M. Notes on the microbiology of cholesteatoma: clinical findings and treatment. *Acta Otorhinolaryngol,* 29 (2009) 197.
6 Haruyama T, Furukawa M, Kusunoki T, Onoda J & Ikeda K, Expression of IL-17 and its role in bone destruction in human middle ear cholesteatoma. *ORL J Otorhinolaryngol Relat Spec,* 72 (2010) 325.
7 Sudhoff H, Dazert S, Gonzales AM, Borkowski G & Park SY, Angiogenesis and angiogenic growth factors in middle ear cholesteatoma. *Am J Otol,* 21 (2000) 793.
8 Raffa S, Leone L, Scrofani C, Monini S & Torrisi MR, Cholesteatoma-associated fibroblasts modulate epithelial growth and differentiation through KGF/FGF7 secretion. *Histochem Cell Biol*, 138 (2012) 251.
9 Olszewska E, Chodynicki S & Chyczewski L, Apoptosis in the pathogenesis of cholesteatoma in adults. *Eur Arch Otorhinolaryngol,* 263 (2006) 409.
10 Bloksgaard M, Svane-Knudsen V, Sorensen JA, Bagatolli L & Brewer J, Structural characterization and lipid composition of acquired cholesteatoma: a comparative study with normal skin. *Otol Neurotol,* 33 (2012) 177.
11 Louw L. Acquired cholesteatoma pathogenesis: stepwise explanations. *J Laryngol Otol,* 124 (2010) 587.
12 Jung HH, Chae SW, Jung SK, Kim ST, Lee HM & Hwang SJ, Expression of a cathelicidin antimicrobial peptide is augmented in cholesteatoma. *Laryngoscope,* 13 (2003) 432.
13 Park K, Moon SK, Choung YH & Choi HS, Expression of beta-defensins in human middle ear cholesteatoma. *Acta Otolaryngol,* 123 (2003) 236.
14 Madsen P, Rasmussen HH, Leffers H, Honore´ B, Dejgaard K & Olsen E, Molecular cloning, occurrence, and expression of a novel partially secreted protein "Psoriasin" that is highly up-regulated in psoriatic skin. *J Invest Dermatol.* 97 (1991) 701.
15 Schroder JM & Harder J, Innate antimicrobial peptides in the skin. *Med Sci (Paris),* 22 (2006) 153.
16 Jinquan T, Vorum H, Larsen CG, Madsen P, Rasmussen HH & Gesser B, Psoriasin: a novel chemotactic protein. *J Invest Dermatol,* 107 (1996) 510.
17 Hoffmann HJ, Olsen E, Etzerodt M, Madsen P, Thøgersen HC & Kruse T, Psoriasin binds calcium and is upregulated by calcium to levels that resemble those observed in normal skin. *J Invest Dermatol*, 103 (1994) 370.
18 Kim KH, Cho J, Song J, Woo J, Lee H, Jung HH, Hwang S & Chae S, Psoriasin (S100A7), an antimicrobial peptide, is increased in human middle ear cholesteatoma. *Acta Oto-Laryngologica,* 29 (2009) 1067.
19 Kobayashi H, Asano K, Kanai K & Suzaki H, Suppressive activity of vitamin D3 on matrix metalloproteinase production from cholesteatoma keratinocytes *in vitro*. *Mediators Inflamm,* 2005 (2005) 210.
20 Suchozebrska-Jesionek D, Szymański M, Kurzepa J, Gołabek W & Stryjecka-Zimmer M, Gelatinolytic activity of matrix metalloproteinases 2 and 9 in middle ear cholesteatoma. *J Otolaryngol Head Neck Surg,* 37 (2008) 628.
21 Schmidt M, Grünsfelder P & Hoppe F, Induction of matrix metalloproteinases in keratinocytes by cholesteatoma debris and granulation tissue extracts. *Eur Arch Otorhinolaryngol*, 257 (2000) 425.

22 Vitale RF & Ribeiro FAQ, The role of tumor necrosis factor-alpha (TNF-α) in bone resorption present in middle ear cholesteatoma. *Braz J Otorhinolaryngol*, 73 (2007) 123.
23 Prinz JC, From bench to bedside-translational research in psoriasis. *J Eur Acad Dermatol Venereol*, 24 (2010) 1.
24 Girolomoni GU, Mrowietz & Paul C, "Psoriasis: rationale for targeting interleukin-17. *Br J Dermatol,* 167 (2012) 717.
25 Nakatsuji T & Gallo RL, Antimicrobial peptides: old molecules with new ideas. *J Invest Dermatol*, 132 (2012) 887.
26 Vegfors J, Ekman SW & Stoll C, Psoriasin (S100A7) promotes stress-induced angiogenesis. *Br J Dermatol,* 75 (2016) 1263.
27 Kumar GN, Pandey SD, Malick S, Gosh SK, Pramanic P & Ghosh AS. Thiol stabilized copper nanoparticles exert antimicrobial properties by preventing cell division in *Escherichia coli. Indian J Biochem Biophys,* 57 (2020) 151.
28 Rana A & Kumar NR. Antioxidative potential of propolis on *Staphylococcus aureus* infected BALC/c mice: A biochemical study. *Indian J Biochem Biophys,* 59 (2022) 1006.