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Cholesteatoma gene expression of matrix metalloproteinases and their inhibitors by RT-PCR

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Abstract

Acquired middle ear cholesteatoma is a benign keratinizing hyperproliferative squamous epithelial lesion that may result in the destruction of the bone structures surrounding the temporal bone. Recent studies show that variations in cellular production of matrix metalloproteinases (MMPs) and their specific inhibitors (TIMPs) contribute to the pathophysiology of cholesteatoma.

Objective: This study aims to analyze the use of RNA amplification tests to evaluate the expression of MMP and TIMP isoforms in cholesteatomas and their correlation with disease severity.

Materials and Methods: This is a prospective study. Nineteen cholesteatoma cases at different stages were selected. RNA collected from biopsy specimens was submitted to reverse transcription polymerase chain reaction (RT-PCR) for semiquantitative amplification of MMP2, MMP3, MMP9, MMP13 and TIMP1.

Results: Six cholesteatomas were positive for at least one of the studied genes. Four samples amplified a single gene (MMP2 or MMP13) and two samples amplified three genes (MMP2, TIMP1 and MMP3 or MMP13). No sample amplified MMP9.

Conclusion: RT-PCR can be used to assess MMP and TIMP gene expression in cholesteatomas despite technical difficulties. Gene expression profiles could not be related to disease severity.

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INTRODUCTION

Middle-ear acquired cholesteatoma is a benign squamous epithelial keratinizing hyperproliferative lesion resembling the epidermis[1] that develops inside the tympanic cavity[2-4]. It manifests as a type of epidermoid cyst with an extracellular matrix made up of squamous stratified keratinized epithelium over a secondary perimatrix containing collagen and elastic fibers, fibroblasts, and inflammatory cells[5,6]. Middle-ear cholesteatomas are characterized by intense cell proliferation - accompanied by the consequent accumulation of keratin debris - and destruction of bone structures surrounding the temporal bone. They may involve the ossicles, blood vessels, the facial nerve, and even invade the inner ear and intracranial space[7-9]. Cholesteatomas may lead to the onset of hearing loss, tinnitus, vertigo, loss of balance, and other severe complications such as meningitis, sigmoid sinus thrombosis, facial paralysis, and brain abscess[2].

It has recently become evident that the proteolytic activity triggered by cholesteatomas plays a key role in the bone remodeling of the middle ear and the temporal bone[10]. Bone lysis and recurrence are relevant features in the pathophysiology of cholesteatoma, giving it the status of a dangerous, difficult-to-treat condition[11]. Alterations in keratinocyte proliferation, differentiation, and migration are impacted by fibroblast activation in the perimatrix and by the release of cytokines and growth factors by inflammatory infiltrate cells[4,12].

Injury to tissues adjacent to cholesteatomas also occurs due to the action of various proteolytic enzymes such as plasminogen activators and matrix metalloproteinases (MMPs)[3,7]. MMPs are zinc and calcium-dependent endopeptidases synthesized by different types of cells such as fibroblasts, keratinocytes, macrophages, and endothelial cells activated by proteolytic cleavage[8,10,13-16]. MMP proteolytic activity is precisely controlled by their precursors during activation and inhibited by endogenous inhibitors, alpha macroglobulins, and tissue inhibitors of metalloproteinases (TIMPs)[8,17]. The balance between MMPs and TIMPs is critical in determining the integrity of the extracellular matrix (ECM); thus, the variations in presence and activity level of these proteins may contribute in a number of tissue events observed in cholesteatoma patients[3,8,17].

Specific cholesteatoma MMP isoenzymes (MMP2, MMP3, and MMP9) were first identified in 1996[17] with the use of immunohistochemistry tests. Since then, immunohistochemistry has been extensively used to analyze and compare changes in enzyme levels in cholesteatomas and healthy tissues. Increased levels of MMP9[7,10,11,14], MMP2[5], MMP1[18], MMP8 and MMP13[19] have been reported. Immune labeling of MMP2, 3 and 9 was observed mainly in the basal and suprabasal layers of the cholesteatoma epithelium[10,14,17]; MMP9 was specifically seen in areas with inflammatory cell infiltration[8].

This study aims to analyze the applicability of reverse transcription polymerase chain reaction (RT-PCR) to semiquantitatively assess gene expression in matrix metalloproteinases and their inhibitors. If RT-PCR is proven adequate, we will attempt to correlate gene expression to disease severity based on the patients' clinical history, audiometric assessment, and imaging findings.

MATERIALS AND METHODS

This is a prospective study designed to offer the basis required to reach the proposed goals and additional information on the topic, aside from aiding in the identification of the determining factors connected to the phenomena and events described herein[20]. Nineteen patients aged between 5 and 70 years diagnosed with various stages of chronic cholesteatomatous otitis media were enrolled in this study. They were seen and operated at the institution's ENT ward from 2007 to 2009. Patients previously submitted to mastoidectomy and with unconfirmed diagnosis of cholesteatoma by pathology tests were excluded. This research project was approved by the Institution's Ethics Committee and granted permit nº. 356/2007.

Auditory involvement was assessed through pure-tone and speech audiometry and ranged from mild conductive dysacusis to profound sensorineural dysacusis. The extension of the involvement by cholesteatoma was analyzed in CT scans and graded based on findings such as scutum erosion, erosion of the ossicular chain, fistula in the lateral semicircular canal, erosion of the tegmen tympani, and Fallopian canal dehiscence. The extent of erosion was assessed by the number of involved structures. Presence of erosion on one structure was categorized as +; erosion on two structures was graded ++; and erosion on three or more structures was rated as +++. None of the patients had advanced stage complications such as facial paralysis, meningitis, encephalitis, or brain abscess.

Open mastoidectomy was the approach used to remove the cholesteatomas in our group of patients. Surgery findings on the extent of involvement and erosion supported the imaging findings on the integrity of the middle ear ceiling (tegmen tympani), the wall of the lateral semicircular canal, the scutum, and the facial canal (Fallopian canal).