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Regular paper

Investigation of EGF, IL1-α and IL-6 levels and selected hematological parameters (NLR, MPV) in patients with the chronic cholesteatomatous otitis media

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In this study we aimed to investigate epidermal growth factor (EGF), interleukin (IL1)-α and IL-6 levels, and hematological parameters in the serum samples of patients with chronic cholesteatomatous otitis media (CCOM). This prospective included 40 patients who underwent surgery due to CCOM between June 2020 and May 2021. The stage of middle ear cholesteatoma was determined on each chart using the EAONO/JOS system. The control group comprised of 30 adults who were scheduled for septoplasty over the same period in our hospital, had no otological complaints, and had normal otological findings. The demographic, clinical, and laboratory data of the patients were obtained from the electronic medical record system of our hospital. The serum EGF, IL1-α and IL-6 levels, and hematological parameters (neutrophil-lymphocyte ratio (NLR) and mean platelet volume (MPV)) were compared between the CCOM and control groups. Seven patients had Stage 1 and 33 patients had Stage 2 middle ear cholestatoma. There was no statistically significant difference between the CCOM and control groups in terms of age and gender (p=0.092 and p=0.616, respectively). The serum EGF and IL1-α levels of the CCOM group were statistically significantly higher than those of the control group (p=0.047 and p=0.013, respectively). No statistically significant difference was observed in the serum IL-6 levels of the CCOM and control groups (p=0.675). There was also no significant difference between the CCOM and control groups in terms of the mean NLR and MPV values (p=0.887 and p=0.164, respectively). There was no significant difference between the Stage 1 and Stage 2 cholesteatoma subgroups in terms of the mean EGF, IL1-α, IL-6 levels (p=0.204, p=0.557 and p=0.613, respectively), and the mean NLR and MPV values (p=0.487, p=0.439, respectively). Increased serum EGF and IL1-α levels in patients with CCOM suggest that these cytokines may play a role in cholesteatomatous epithelial hyperproliferation.

Keywords: cholesteatoma, chronic otitis media, EGF, IL1-α, IL-6, NLR, MPV

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Abbreviations: CCOM, chronic cholesteatomatous otitis media; COM, chronic otitis media; EGF, Epidermal growth factor; IL, interleukin; MPV, mean platelet volume; NLR, neutrophil-lymphocyte ratio; TP, total protein

INTRODUCTION

Cholesteatoma is characterized by the coexistence of a keratinized epithelium with subepithelial immune cell infiltration, which causes severe bone resorption (Bujia *et al.*, 2003; Palva, 1990). Most inflammatory cells are lymphocytes and macrophages, both in an immunologically active state, suggesting that cholesteatoma inflammation is an immune-mediated process. Histopathological changes include the hyperproliferation and differentiation of the epithelium, accompanied by bone resorption.

Recent findings suggest a link between processes that occur in the middle ear during otitis and cytokine levels (Skovbjerg *et al.*, 2010). Cytokines control the durability of immune and inflammatory responses by accelerating cell growth, activation and differentiation and antibody production (Schilling *et al.*, 1991; Warren & Stephen, 1988). Bacterial otitis media is caused by the migration of pathogens from the nasopharynx to the middle ear, considering that the endotoxin in the bacterial cell wall component causes the initiation of inflammation in the middle ear. The endotoxin is a modulator of the immune response, stimulates macrophages to produce tumor necrosis factor α (TNF-α) and IL-1β (Juhn *et al.*, 2008). Keratinocytes produce mediators of inflammation such as IL-1 α and IL-1β, IL-6 and IL-8 (Akimoto *et al.*, 2000, Juhn *et al.*, 2008).

In cholesteatoma, epithelial proliferation is rapid (Tanaka *et al.*, 1998). Cytokines and epidermal growth (EGF) factor have a significant effect on epithelial proliferation (Bujia *et al.*, 1997; Carpenter *et al.*, 1986). EGF is a potent stimulator of cell proliferation and differentiation. EGF activates tyrosine protein kinase, which induces mitotic activity by binding to the receptor located on the cell surface (Bujía *et al.*, 1993). Yetişer *et al* showed that cholesteatoma epithelium has a high concentration of EGF, which makes it more invasive than normal epithelium (Yetişer *et al.*, 2002). Interleukin (IL)-1, originally called lymphocyte-activating factor due to its stimulating effect on T lymphocytes, plays an important role in cholesteatoma-related bone resorption through the activation of osteoclasts and stimulation of fibroblasts and other inflammatory cells (Ahn *et al.*, 1990; Schilling *et al.*, 1992). IL-1 is mainly released from monocytes and macrophages and binds to cell surface receptors (Mundy, 1993; Ottaviani *et al.*, 1999). On the other hand, it has been shown that keratinocytes can also produce IL-1 (Ahn *et al.*1990; Kım *et al.*, 1996). In a study by Serban *et al.*, the authors recorded high serum levels of IL-1α, IL-6, and IL-8 in all otitis media groups compared to the healthy group. In the same study, IL-1α had the highest

value in patients with cholesteatoma recidivism (Serban *et al*., 2021). IL-6 is a cytokine produced by various cells and triggers cell proliferation and differentiation. It exhibits its effects mostly through lymphocytes, fibroblasts, keratinocytes, and hepatocytes (Bujía *et al*., 1996, Hirano *et al*., 1990; Van Snick, 1990).

The diagnosis of cholesteatoma is based on an otological examination and radiological imaging, and there is currently no hematological marker for its diagnosis. However, some parameters routinely measured in complete blood count analysis have recently become popular as inflammatory, proinflammatory, and prothrombotic markers. The neutrophil-lymphocyte ratio (NLR) can be used as a systemic inflammatory marker since it reflects both the increase in neutrophils and the decrease in lymphocytes (Yenigun *et al*., 2015, Aslan *et al*., 2021). The mean platelet volume (MPV) can also be used as a marker of inflammation by measuring the volume of circulating platelets with an increased MPV indicating the presence of more intense inflammation (Gasparyan *et al*., 2011, Eryilmaz *et al*., 2016, Aslan *et al*., 2021).

In this study, we investigated the serum levels of EGF, IL1-α, and IL-6 in patients with acquired chronic cholesteatomatous otitis media (CCOM) using an immunoassay method to clarify the possible role of these parameters in the pathogenesis of middle ear cholesteatoma. In addition, we calculated the hematological parameters of NLR and MPV. We then compared the CCOM and control groups in terms of their serum cytokine levels and hematological parameters.

MATERIALS AND METHODS

This prospective study included 40 patients who underwent surgery due to CCOM between June 2020 and May 2021. The control group comprised of 30 adults who were scheduled for septoplasty over the same period in our hospital, had no otological complaints, and had normal otological findings.

The demographic, clinical, and laboratory data of the patients were obtained from the electronic medical record system of our hospital. None of the patients or controls had diabetes mellitus, acute or chronic renal failure, chronic liver disease, asthma, connective tissue disease, inflammatory systemic disease, or pneumonia. Patients with a history of otologic surgery, those with congenital cholesteatoma, those with complications, and those under 18 and over 65 years were not included in the study.

The diagnosis of chronic otitis media (COM) was based on the presence of a perforated tympanic membrane and ear discharge that had lasted for more than three months. The diagnosis of cholesteatoma was based on the observation of the cholesteatoma matrix during surgery and the histopathological confirmation of cholesteatoma. The stage of middle ear cholesteatoma was determined on each chart using the EAONO/JOS system (Yung *et al*., 2017). According to this classification, seven patients had Stage 1 and 33 patients had Stage 2 middle ear cholestatoma.

Our main indicators for canal wall-down tympanomastoidectomy (CWDTM) were extensive cholesteatoma advancing into the mastoid and beyond, eustachian tube (anteromedial to the ossicles) or ST, extensive damage of the external auditory canal by disease, failure of previous canal wall-up surgery with recurrent cholesteatoma from epitympanic retraction pockets, patients with poor preoperative auditory thresholds, complicated cases, and the patients whose postoperative follow-up constitutes a problem.

Venous blood, 10 mL, was taken from the individuals in the CCOM and control groups and divided into two tubes. The first tube contained ethylene diamine tetra acetic acid to evaluate the complete blood count. Complete blood count analyses were automatically performed using Sysmex XN-3000 (Kobe, Japan). The neutrophil, lymphocyte, thrombocyte, and MPV values were recorded from the results of these analyses. NLR was calculated as a simple ratio between the absolute neutrophil and absolute lymphocyte counts. The second tube was left in an incubator for 30 minutes, centrifuged at 3000 rpm for 10 minutes, and then the separated serum was stored at –80°C until the end of the study. IL1-α, IL-6, and EGF measurements were performed using the MRC UT6100 ELISA reader with the Elabscience human IL1-α (Cat.No.:E-EL-H0088), Elabscience human IL-6 (Cat.No.:E-EL-H6156), and Elabscience human EGF (Cat.No:E-EL-H0059) ELISA kits, respectively. The results were recorded as pg/ml for all the three parameters. The intra-assay and inter-assay % CV values were reported as <5.3 and <5.81, respectively for IL1-α, <4.15 and <5.11, respectively for IL-6, and <5.4 and <5.82, respectively for EGF.

The serum EGF, IL1-α and IL-6 levels, and hematological parameters (NLR and MPV) were compared between the CCOM and control groups.The study was approved by the local ethics committee and informed consent was obtained from all the participants. The study was carried out in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

Statistical analyses were performed using PASW version 19.0 software (SPSS Inc., Chicago, IL, USA). Descriptive statistics were shown as mean±standard deviation (S.D.). The Shapiro-Wilk test was used to check the normality of the data. The chi-square and Fisher's exact tests were employed for the comparison of categorical data between groups. The independent samples t-test was conducted to compare the serum EGF, IL1-α and IL-6 levels, and hematological parameters between groups. *P* values of less than 0.05 were considered statistically significant for all tests.

RESULTS

Thirty patients (EANOS/JOS Stage 2) underwent CWDTM and 10 patients (7 patients, EANOS/JOS Stage 1; 3 patients, EANOS/JOS Stage 2) underwent canal wall-up tympanomastoidectomy. The demographic and laboratory data of the CCOM and control groups are presented in Table 1. The mean EGF and IL1-α levels were statistically significantly higher in the CCOM group compared to the control group (p=0.047 and p=0.013, respectively). The mean IL-6 level of the CCOM group was higher than that of the control group, but this difference was not statistically significant (p=0.675). There was also no significant difference between the CCOM and control groups in terms of the mean NLR and MPV values (p=0.887 and p=0.164, respectively).

There was no significant difference between the Stage 1 and Stage 2 cholesteatoma subgroups in terms of the mean EGF, IL1-α, IL-6 levels (p=0.204, p=0.557 and p=0.613, respectively), and the mean NLR and MPV values (p=0.487, p=0.439, respectively) (Table 2).

Table 1. Demographic characteristics and laboratory data of the CCOM and control groups

	CCOM (40)	Control (30)	*p*
Age	42.9±14.3	37.2±14.1	0.092
Gender (F/M)	27/13	18/12	0.616
EGF (pg/ml)	93.3±72.1	59.7±53.7	0.047
IL-1-α (pg/ml)	359.8±282.6	86.8±44.5	0.013
IL-6 (pg/ml)	66.4±54.8	58.9±50.1	0.675
NLR	2.4±1.1	2.4±1.3	0.887
MPV (fl)	8.57±0.99	8.34±0.78	0.164

CCOM, chronic cholesteatomatous otitis media; F, female; M, male; EGF, epidermal growth factor; IL, interleukin; NLR, neutrophil-lymphocyte ratio; MPV, mean platelet volume

Table 2. Comparison of EGF, IL-1-α, IL-6 levels and hematological parameters between Stage 1 and Stage 2 cholesteatoma subgroups

	Stage 1 (7)	Stage 2 (33)	*p*
EGF (pg/ml)	92.6±106.8	94.3±60.1	0.204
IL-1-α (pg/ml)	349.8±82.6	361.8±74.5	0.557
IL-6 (pg/ml)	72.4±50.8	65.9±50.1	0.613
NLR	2.3±1.9	2.5±0.8	0.487
MPV (fl)	8.45±0.44	8.58±0.78	0.439

EGF, epidermal growth factor; IL, interleukin; NLR, neutrophil-lymphocyte ratio; MPV, mean platelet volume

DISCUSSION

As a main finding of this study, we found that EGF and IL-1α were non-invasive and reliable independent indicators of cholesteatoma in adult patients with CCOM. On the other hand, we also determined that NLR and MPV were not independent indicators of adult CCOM.

Cholesteatoma consists of a hyperproliferative, keratinized epithelium and is defined as a chronic inflammatory, destructive, and lytic middle ear/mastoid disorder (Al-Ani & Dhia'a, 2012; Peek *et al*., 2003). The production of inflammatory cytokines and bioactive molecules, such as IL-1, PAF, TNF -α, and neutrophil proteases is triggered by bacterial toxins and lipopolysaccharides produced by gram-negative bacteria and by keratinocytes in the presence of cholesteatoma (Al-Ani & Dhia'a, 2012; Peek *et al*., 2003; Yetiser *et al*., 2002). These cytokines lead to the hyperproliferation of epithelial cells, mucin hypersecretion, and bone resorption with the activation of collagenases through the stimulation of macrophages and osteoclasts (Yetiser *et al*., 2002; Masanta *et al*., 2015). Recurrent infections and chronic inflammation arising from the perimatrix of cholesteatoma are the most important factors for the expanding growth of cholesteatoma (Yetiser *et al*., 2002; Nagai *et al*., 2007). If CCOM is not treated it can cause ossicular chain destruction, vestibular dysfunction, facial paralysis, and intracranial complications due to bone erosion caused by chronic inflammation (Baysal *et al*., 2013). Therefore, early diagnosis and treatment are important to prevent these complications.

Yetiser *et al*. determined the mean EGF level as 472.43±324.53 ng/mg total protein (TP) in samples taken from cholesteatoma tissues in patients with CCOM, and they stated that this value was significantly higher compared to the EGF level of the granulation tissue samples of the COM group (158.84±242.63 ng/mg TP) and that of the external ear canal tissue samples of the control group (120.58±211.60 ng/mg TP) (Yetiser *et al*., 2002). In the current study, the mean serum EGF level was significantly higher in the CCOM group compared to the control group. Our results are consistent with the results of the above-mentioned studies, and it can be suggested that the EGF signaling pathway is active in the cholesteatoma epithelium and may represent a new target for intratympanic drug therapy.

IL1-α, released from keratinocytes and macrophages, initiates bone resorption by activating osteoclasts and bone resorption enzymes (Schilling *et al*., 1992). Bujia *et al*. observed increased epithelial proliferation as a result of high IL-1 receptor levels in the middle ear cholesteatoma (Bujia *et al*., 1997). Yetiser *et al*. showed that the IL1-α level was higher in the cholesteatoma tissue samples of the patients with COM compared to the granulation tissue samples of the same group and the external ear canal tissue samples of the control group (Yetiser *et al*., 2002). In our study, the mean serum IL1-α level of the CCOM group was found to be significantly higher than that of the control group. All these results emphasize the importance of IL1-α in the pathogenesis and development of cholesteatoma.

IL-6 is a proinflammatory cytokine characterized as a potent activator of STAT3. IL-6 and STAT3 function cooperatively to promote cellular proliferation and inhibit apoptosis (Chang *et al*., 2013). In an immunohistochemical study evaluating patients with CCOM, Liu *et al*. determined that IL-6 expression was 72% in the cholesteatoma tissue samples and 20% in the normal ears (Liu *et al*., 2014). Similarly, Edward *et al*. stated that IL-6 expression was four times higher in the ears with CCOM compared to the healthy ears (Edward *et al*., 2019). In our study, the serum IL-6 level was higher in patients with CCOM compared to the control group, but this difference was not statistically significant. However, the interpretation of this result is limited due to the lack of a comparative analysis of the serum IL-6 levels of the CCOM and control groups in the literature.

NLR and MPV have been accepted as systemic inflammatory markers and predictors of poor clinical outcomes for many diseases (Tamhane *et al*., 2008; Halazun

et al., 2008; Arruda-Olson *et al*., 2009; Gibson *et al*., 2007; Cedres *et al*., 2012; Tansuker *et al*., 2017). NLR has been investigated in various otolaryngological diseases, including malignancies, obstructive sleep apnea syndrome, Bell's palsy, sudden hearing loss, tinnitus, adenoidectomy, and tonsillectomy (Yiğit *et al*., 2018). NLR has also been studied in patients with COM to detect the effects of this disease on inflammatory blood parameters. Tansuker *et al*. investigated the predictive value of NLR in distinguishing between active and inactive COM and concluded that this parameter did not help differentiate between the two (Tansuker *et al*., 2017). Kilickaya *et al*. investigated the systemic inflammatory effect of CCOM and showed that NLR had no predictive value in terms of bone erosion and related complications in patients with cholesteatoma (Kılıckaya *et al*., 2017). Similarly, in the same study, no significant difference was found between the COM, CCOM, and control groups in terms of the NLR and MPV values. Consistently, we observed no significant difference between the CCOM and control groups in terms of NLR and MPV.

To our knowledge, the comparative analysis of the serum EGF, IL1-α and IL-6 levels between the CCOM and control groups has not been previously undertaken. The feature that distinguishes our study from the other studies in the literature is that we examined cytokine levels in the serum samples rather than in the cholesteatoma tissue samples of patients with CCOM. We consider that this may have prevented cytokine level differences that could occur depending on the amount of tissue studied. The main limitations of our study are the small number of patients included in our study and the absence of follow-up data after cholesteatoma surgery. Further prospective studies with a larger number of patients are needed on this subject.

In conclusion, increased serum EGF and IL1-α levels in patients with CCOM suggest that these cytokines may play a role in cholesteatomatous epithelial hyperproliferation. We suggest that anti-EGF and IL1-α therapies may function as potent antiproliferative agents in cholesteatoma.

Conflict of interest

All authors declare no conflict of interest.

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