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Expression of Toll-like receptor-9, CD28, and CD152 in chronic suppurative Otitis media evidence for innate-adaptive immune crosstalk in bacterial persistence

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Abstract

Objective

Chronic suppurative otitis media (CSOM) is a long-term inflammatory disease in the middle ear. This disease is associated with different factors. Some of these factors are persistent infection, tissue damage, and bacterial biofilm formation. This situation indicates that the immune status has changed. This condition allows bacteria to survive and continue to cause inflammation. One of the most important regulatory pathways is the CD28-CD152 (CTLA-4) axis. CD28 provides activating signals. These signals promote T-cell activation, proliferation, and cytokine production. On the other hand, CD152 is an inhibitory receptor. It suppresses T-cell responses through competing with CD28 for B7 ligands. Moreover, Toll-like receptor 9 (TLR-9) is also involved in innate immune responses. This receptor recognizes bacterial DNA and contributes to chronic inflammation. The aim of this study was to evaluate the serum levels of TLR-9, CD28, and CD152 in patients with CSOM and to examine their relationship with bacterial infection patterns.

Materials and methods

Fifty patients with CSOM and twenty-five healthy controls were used in this study. Individuals who had persistent ear discharge for more than three months and whose tympanic membrane

rupture was confirmed by otoscopic and radiological examinations were considered to have CSOM. Ear swab samples were collected from patients. Then cultured for bacterial isolation. The VITEK 2 Compact system was used to identify bacteria. Enzyme-linked immunosorbent assay (ELISA) was used to measure serum levels of TLR-9, CD28, and CD152 in blood samples of all participants.

Results

The most common bacteria isolated in this study were *Staphylococcus aureus* (38%), *Pseudomonas aeruginosa* (26%), *Proteus mirabilis* (14%), *Klebsiella pneumoniae* (12%), and *Escherichia coli* (10%). Serum TLR-9 levels were significantly higher in CSOM patients compared with controls (1.62 ± 0.07 ng/mL vs 0.48 ± 0.05 ng/mL; $P < 0.001$). CD28 levels were significantly lower in patients than in controls (0.42 ± 0.09 ng/mL vs 1.18 ± 0.06 ng/mL; $P < 0.001$). In contrast, CD152 levels were significantly increased in CSOM patients (1.33 ± 0.11 ng/mL vs 0.26 ± 0.08 ng/mL; $P < 0.001$). A significant positive correlation was observed between both *S. aureus* infection and TLR-9 expression ($r = 0.69$), and between *P. aeruginosa* infection and increased CD152 levels ($r = 0.61$).

Conclusion

The results of this study showed that CSOM is associated with both increased expression of TLR-9 and CD152 and decreased levels of CD28. It can be concluded that this immune pattern may increase immune tolerance, bacterial persistence, and chronic inflammation in CSOM.

Keywords: Chronic suppurative otitis media, immune modulation, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Toll-like receptor 9

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Introduction

One of the most common and disabling forms of middle ear infection in developing countries is Chronic suppurative otitis media (CSOM). This condition usually occurs when the eardrum is perforated and chronic inflammation of the mucoperiosteum lining the middle ear cavity occurs and is accompanied by persistent or recurrent ear discharge (Verhoeff et al., 2006). CSOM shows a complex disease procedure that results from extended interaction between host immune responses and pathogenic microorganisms. A key characteristic of this situation is the building of bacterial biofilms. It protects pathogens from antimicrobial factors and immune-mediated release. So, it contributes to disease application (Hall-Stoodley et al., 2006). Among the creator factors, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most frequently isolated bacteria in CSOM. These pathogens have a big ability to form biofilms and produce various virulence factors. They include proteases and exotoxins which increase tissue damage and maintain chronic inflammation (Ibrahim et al., 2022). Other bacterial species, such as *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Escherichia coli*, are considered secondary or opportunistic pathogens. They have also been associated with persistent mucosal injury in CSOM (Palusiak, 2022). In addition to bacterial persistence, immune dysregulation plays a major role in the chronic course of CSOM. Toll-like receptors (TLRs) are essential components of the innate immune system. They recognize pathogen-associated molecular patterns (PAMPs). Among them, TLR-9 detects unmethylated CpG motifs in bacterial DNA. Thus, it activates nuclear factor kappa B (NF-κB) and type I interferon signaling pathways. This activation leads to cytokine production and macrophage activation (Akira et al., 2006). However, continuous stimulation of TLR-9 may contribute to immune exhaustion and sustained inflammation. So, it further supports chronic infection (Lee et al., 2013). Adaptive immune responses are also altered in CSOM, particularly via changes in co-stimulatory signaling pathways. The CD28-CD152 (CTLA-4) axis plays a central role in regulating T-cell activity and immune homeostasis. CD28 provides activating signals. This signal requires for T-cell activation, proliferation, and cytokine secretion. In contrast, CD152 (CTLA-4) is an inhibitory receptor. This receptor suppresses T-cell responses by competing with CD28 for binding to B7 ligands (Esensten et al., 2016; Rowshanravan et al., 2018). In chronic infections, sustained expression of CD152 and reduced expression of CD28 are commonly observed. It reflects T-cell exhaustion and immune tolerance toward persistent antigens (Chihara et al., 2018). In CSOM, these innate and adaptive immune mechanisms create an immunological environment. It characterizes by a balance between activation and inhibition. This allows the bacteria to remain in this state. Therefore, it keeps inflammation at a controlled level and prevents excessive tissue destruction. However, this immune balance cannot completely eliminate the bacteria. Therefore, persistent infection occurs (Mittal et al., 2014). Therefore, the aim of this study was to evaluate

the expression levels of TLR-9, CD28, and CD152 in patients with chronic suppurative otitis media. The goal of this research was also to determine their expression levels with the bacterial pathogens isolated from middle ear discharge.

Materials and methods

Study design: This analytical case-control study was designed to investigate the association between bacterial pathogens and immune regulatory biomarkers (TLR-9, CD28, and CD152) in patients with chronic suppurative otitis media (CSOM). This study design was chosen because it allows comparison of immunological parameters between affected patients and healthy individuals (Verani et al., 2017).

Study population: The study included 50 patients clinically diagnosed with CSOM and 25 apparently healthy individuals as a control group. The diagnosis of CSOM was based on a history of continuous ear discharge for more than three months through a perforated tympanic membrane. Diagnosis was confirmed by otoscopic examination and radiological findings (Shwetha, 2018). The inclusion criteria were individuals aged between 15 and 70 years. The exclusion criteria included patients with acute otitis media, known systemic autoimmune diseases, or those receiving immunosuppressive therapy (Mittal et al., 2014). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Sample collection and processing: Middle ear discharge samples were collected aseptically from the external auditory canal using sterile cotton swabs. Each specimen was immediately placed in Amies transport medium and delivered to the microbiology laboratory within two hours for bacterial culture (Cruickshank, 1974). At the same time, 5 mL of venous blood was collected from each participant. Blood samples were centrifuged at 3000 rpm for 10 minutes to separate the serum. The sera were stored at −20°C until immunological analysis was performed (Tuck et al., 2009).

Bacterial isolation and identification: Collected samples were inoculated onto blood agar, MacConkey agar, and mannitol salt agar plates. The plates were incubated aerobically at 37°C for 24 to 48 hours. Bacterial isolates were initially identified based on colony morphology, Gram staining, and standard biochemical tests, including catalase, oxidase, indole, citrate, urease, and triple sugar iron reactions (Cheesbrough, 2010). Final identification and antibiotic profile confirmation were performed using the VITEK 2 Compact system (bioMérieux, France) according to the manufacturer's instructions (Ligozzi et al., 2002). The identified bacteria included *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Escherichia coli*.

Measurement of immune markers: Serum levels of TLR-9, CD28, and CD152 (CTLA-4) were measured using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, USA). All assays were performed according to the manufacturer's protocols and previously described methods (Lancaster et al., 2005; Schmidt & Sester, 2013). Briefly, 100 µL of serum was added to microtiter plates pre-coated with monoclonal antibodies specific to each marker. After incubation with biotinylated detection antibodies and streptavidin-HRP conjugate, color development was achieved using TMB substrate. Optical density was measured at 450 nm using a BioTek ELX-808 microplate reader. Concentrations were calculated from standard curves using a four-parameter logistic regression model.

Correlation with bacterial isolates: For each patient, serum immune marker levels were compared with the corresponding bacterial culture results. Pearson correlation coefficients (r) were calculated to evaluate the association between specific bacterial species and immune markers, particularly between *S. aureus* and TLR-9 levels, and between *P. aeruginosa* and CD152 expression (Nokso-Koivisto et al., 2024).

Statistical analysis: Statistical analysis was performed using IBM SPSS software version 26. Data were expressed as mean ± standard error (SE). An independent samples t-test was used to compare differences between patients and controls. Pearson correlation analysis was applied to assess relationships between immune marker levels and bacterial isolates. A P value of less than 0.05 was considered statistically significant (Field, 2018).

Results

Bacterial isolation and distribution: Ear discharge samples from patients with chronic suppurative otitis media (CSOM) were cultured on selective and differential media and incubated at 37°C for 18-24 hours. Bacterial identification was performed based on colony morphology, microscopic examination, and standard biochemical tests. On mannitol salt agar, *Staphylococcus aureus* produced yellow colonies with a surrounding yellow halo. *Klebsiella pneumoniae* and *Escherichia coli* appeared as lactose-fermenting pink colonies on MacConkey agar, with *K. pneumoniae* showing large, mucoid colonies. In contrast, *Pseudomonas aeruginosa* formed non-lactose-fermenting, pale, flat colonies with irregular margins and a slight greenish pigment on MacConkey agar (Figure 1). Bacteriological analysis of ear discharge samples from 50 CSOM patients showed polymicrobial infection, with *S. aureus* and *P. aeruginosa* as the predominant isolates. As shown in Table 1, *S. aureus* was the most frequently isolated pathogen (38%), followed by *P. aeruginosa* (26%), *Proteus mirabilis* (14%), *Klebsiella pneumoniae* (12%), and *E. coli* (10%). No bacterial growth was detected in samples from the healthy control group.

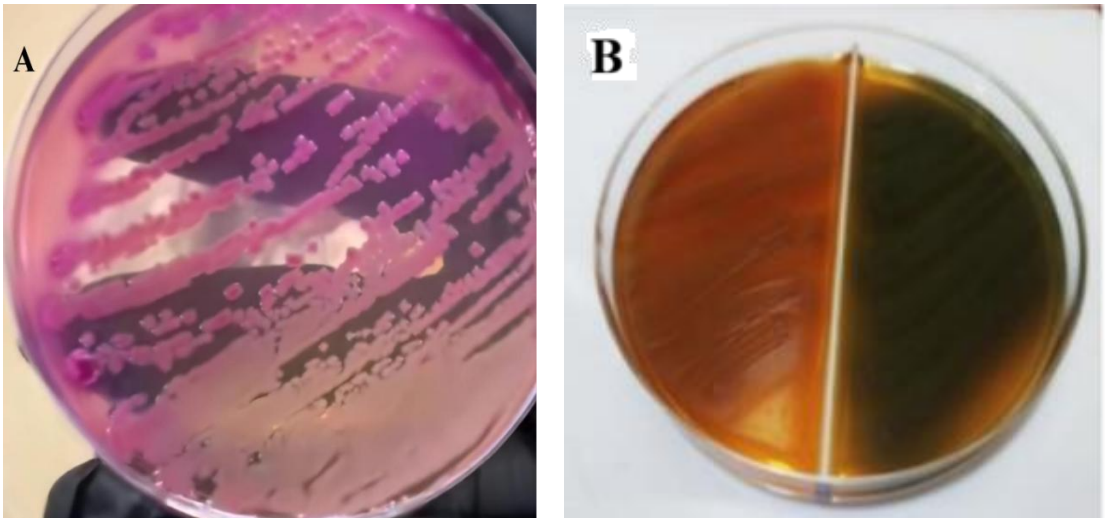

Figure 1. Colony morphology of bacterial isolates from middle ear discharge samples of CSOM patients. (A) *Klebsiella pneumoniae* showing large, mucoid lactose-fermenting colonies on MacConkey agar. (B) *Pseudomonas aeruginosa* showing pale, non-lactose-fermenting colonies with irregular margins on MacConkey agar

Table 1. Bacterial isolates identified from ear discharge samples of CSOM patients

Bacterial species	Number of isolates (n = 50)	Percentage (%)
Staphylococcus aureus	19	38.0
Pseudomonas aeruginosa	13	26.0
Proteus mirabilis	7	14.0
Klebsiella pneumoniae	6	12.0
Escherichia coli	5	10.0
Total	**50**	**100.0**

Serum TLR-9 levels: Serum concentrations of TLR-9 were significantly higher in CSOM patients than in healthy controls. The mean TLR-9 level in patients was 1.62 ± 0.07 ng/mL, while the mean level in controls was 0.48 ± 0.05 ng/mL. This difference was statistically significant ($t = 9.84$, $P < 0.001$), as shown in Table 2.

Table 2. Serum TLR-9 levels in CSOM patients and healthy controls

Group	Number of subjects (n)	Mean ± SE (ng/mL)	t-value	P-value
CSOM patients	50	1.62 ± 0.07	9.84	< 0.001
Controls	25	0.48 ± 0.05	-	-

CD28 expression: Serum levels of CD28 were significantly lower in CSOM patients compared with the control group. The mean CD28 concentration in patients was 0.42 ± 0.09

ng/mL, whereas controls showed a mean level of 1.18 ± 0.06 ng/mL. This reduction was statistically significant (t = 8.02, P < 0.001) (Table 3).

Table 3. Serum CD28 levels in CSOM patients and healthy controls

Group	Number of subjects (n)	Mean ± SE (ng/mL)	t-value	P-value
CSOM patients	50	0.42 ± 0.09	8.02	< 0.001
Controls	25	1.18 ± 0.06	-	-

CD152 (CTLA-4) expression: Serum CD152 (CTLA-4) levels were significantly increased in CSOM patients compared with healthy controls. Patients showed a mean CD152 concentration of 1.33 ± 0.11 ng/mL, while controls had a mean value of 0.26 ± 0.08 ng/mL. The difference between the two groups was statistically significant (t = 7.44, P < 0.001), as presented in Table 4.

Table 4. Serum CD152 (CTLA-4) levels in CSOM patients and healthy controls

Group	Number of subjects (n)	Mean ± SE (ng/mL)	t-value	P-value
CSOM patients	50	1.33 ± 0.11	7.44	< 0.001
Controls	25	0.26 ± 0.08	-	-

Correlation between bacterial isolates and immune markers: Correlation analysis revealed a significant positive association between *Staphylococcus aureus* infection and serum TLR-9 levels (r = 0.69, P < 0.001). In addition, *Pseudomonas aeruginosa* infection showed a significant positive correlation with increased CD152 expression (r = 0.61, P < 0.001). No statistically significant correlations were observed between other bacterial species and CD28 levels (Table 5).

Table 5. Correlation between bacterial isolates and immune marker expression in CSOM patients

Bacterial species	Immune marker	Correlation coefficient (r)	P-value	Interpretation
Staphylococcus aureus	TLR-9	0.69	< 0.001	Strong positive correlation
Pseudomonas aeruginosa	CD152	0.61	< 0.001	Strong positive correlation
Proteus mirabilis	CD28	-0.24	> 0.05	Weak negative correlation
Klebsiella pneumoniae	TLR-9	0.31	> 0.05	Mild positive trend
Escherichia coli	CD152	0.28	> 0.05	Mild positive trend

Summary of immune marker profiles: A comparative analysis of immune markers revealed a distinct immunological pattern in CSOM patients. TLR-9 and CD152 levels were significantly upregulated, while CD28 levels were significantly downregulated compared with controls (Table 6). This combined immune profile indicates persistent innate immune activation together with enhanced inhibitory signaling in the adaptive immune response. Overall, the results demonstrate that CSOM is associated with persistent bacterial infection and altered immune regulation. The predominance of *S. aureus* and *P. aeruginosa* coincided with increased TLR-9 and CD152 expression and reduced CD28 levels, which may contribute to immune tolerance and chronic infection.

Table 6. Comparison of immune marker levels between CSOM patients and healthy controls

Immune marker	Mean ± SE (Patients)	Mean ± SE (Controls)	Direction of change	P-value	Immunological implication
TLR-9	1.62 ± 0.07	0.48 ± 0.05	↑ Increased	< 0.001	Persistent innate activation
CD28	0.42 ± 0.09	1.18 ± 0.06	↓ Decreased	< 0.001	Reduced T-cell activation
CD152 (CTLA-4)	1.33 ± 0.11	0.26 ± 0.08	↑ Increased	< 0.001	Enhanced inhibitory regulation

Discussion

The present study provides clear evidence that bacterial persistence in chronic suppurative otitis media (CSOM) is closely associated with dysregulation of both innate and adaptive immune responses. This immune imbalance is characterized by increased expression of TLR-9 and CD152 (CTLA-4) and a marked reduction in CD28 expression. Together, these changes create an immune environment that allows bacteria to persist within the chronically inflamed middle ear mucosa. The bacteriological findings of this study showed that *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the predominant pathogens in CSOM patients. This pattern is consistent with previous reports identifying these organisms as the main causative agents of CSOM (Hiremath et al., 2018; Alam et al., 2022). Both bacteria have a strong ability to form biofilms, which protect them from antimicrobial agents and immune clearance. Biofilms also act as a continuous source of microbial components that stimulate immune receptors. In particular, *S. aureus* biofilms release unmethylated CpG DNA motifs that strongly activate TLR-9 signaling (Li et al., 2023). Similarly, *P. aeruginosa* produces exopolysaccharides and quorum-sensing molecules that modify host immune responses and support bacterial persistence (Kariminik et al., 2017). In this study, serum

TLR-9 levels were significantly elevated in CSOM patients compared with healthy controls. This finding suggests persistent activation of innate immunity due to continuous exposure to bacterial DNA and biofilm-related products. Activation of TLR-9 triggers downstream MyD88-dependent signaling, leading to NF-κB activation and production of pro-inflammatory cytokines such as TNF-α and IL-1β (Huang & Yang, 2010). While this response is necessary for pathogen recognition, prolonged stimulation can result in immune exhaustion and sustained inflammation. Similar increases in TLR-9 expression have been reported in other chronic inflammatory diseases, including chronic rhinosinusitis and bronchiectasis, where microbial persistence maintains a cycle of inflammation (Fastenberg et al., 2018). In addition to innate immune activation, significant alterations were observed in adaptive immune regulation. CD28 expression was markedly reduced in CSOM patients, indicating impaired T-cell activation and proliferation. CD28 is essential for T-cell co-stimulation, interleukin-2 production, and effective cytotoxic responses. Reduced CD28 expression is a recognized feature of chronic antigen exposure and T-cell exhaustion (Humblin et al., 2023). Similar findings have been reported in chronic bacterial infections such as *Helicobacter pylori* gastritis and chronic periodontal disease (Saeidi et al., 2018). Conversely, CD152 (CTLA-4) expression was significantly increased in CSOM patients and showed a strong positive correlation with *P. aeruginosa* infection. CD152 is a potent inhibitory receptor that competes with CD28 for B7 ligands and suppresses T-cell activity (Hossen et al., 2023). Persistent upregulation of CD152 has been associated with immune tolerance in chronic infections and may act as a compensatory mechanism to limit excessive inflammation (Chang et al., 2025) [32]. However, this inhibitory dominance may also reduce effective bacterial clearance and promote disease chronicity. The combined pattern of increased TLR-9 and CD152 expression with decreased CD28 suggests coordinated crosstalk between innate and adaptive immune pathways. Persistent TLR-9 activation may indirectly enhance CTLA-4 expression through cytokine-mediated signaling, creating a feedback loop that suppresses adaptive immunity (Duan et al., 2022). Similar immune profiles have been described in chronic pulmonary and gastrointestinal infections, where sustained innate activation coexists with adaptive immune suppression (Wang et al., 2020). Clinically, these findings highlight the importance of immune checkpoint and TLR-mediated pathways in CSOM pathogenesis. Circulating TLR-9 and CD152 levels may serve as potential biomarkers for disease chronicity and treatment response. Therapeutic strategies that restore CD28 signaling or modulate CTLA-4 activity may help improve immune function and bacterial clearance. However, due to the sensitive anatomy of the middle ear, localized immunomodulatory approaches should be considered to avoid excessive inflammation (Paluch et al., 2018).

Conclusions: In conclusion, this study demonstrates that CSOM is maintained by a dual immune mechanism involving persistent innate immune activation and concurrent suppression of adaptive immunity. The coordinated modulation of TLR-9, CD28, and CD152 contributes to bacterial persistence, chronic inflammation, and failure of sterilizing immunity. This immune marker triad may provide valuable insight into disease mechanisms and offer new targets for therapeutic intervention in chronic suppurative otitis media.

Author Contributions

I.N.K. and M.A.K.A. contributed to the conceptualization and design of the study. They were responsible for methodology development, data collection, formal analysis, data curation, and drafting the original and revised versions of the manuscript. S.H.A. contributed to supervision and visualization. All authors reviewed and approved the final version of the manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Ethical approval

This study was approved by the Ethical Approval Committee of the College of Education, University of Babylon, Iraq (Reference No. 318, dated 7/4/2024). All procedures were conducted in accordance with ethical standards, and informed consent was obtained from all participants. The study was performed with scientific integrity and without fabrication, falsification, plagiarism, or any form of scientific misconduct.

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Conflict of interest

The authors declare that they have no competing interests related to this study.

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بیان گیرنده Toll-like receptor-9، CD28 و CD152 در اوتیت میانی چرکی مزمن: شواهدی از تعامل ایمنی ذاتی-اکتسابی در پایداری باکتری‌ها

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چکیده

هدف: اوتیت میانی چرکی مزمن (CSOM) یک بیماری التهابی طولانی‌مدت گوش میانی است که با عوامل متعددی همراه می‌باشد. از جمله این عوامل می‌توان به عفونت پایدار، آسیب بافتی و تشکیل بیوفیلم‌های باکتریایی اشاره کرد. این وضعیت نشان‌دهنده تغییر در وضعیت سیستم ایمنی است، به‌گونه‌ای که امکان بقای باکتری‌ها و تداوم التهاب فراهم می‌شود. یکی از مهم‌ترین مسیرهای تنظیمی در این فرآیند، محور CD28-CD152 (CTLA-4) است. CD28 سیگنال‌های فعال‌کننده‌ای را فراهم می‌کند که موجب فعال‌سازی، تکثیر سلول‌های T و تولید سایتوکاین‌ها می‌شود. در مقابل، CD152 یک گیرنده مهاری است که از طریق رقابت با CD28 برای لیگاندهای B7، پاسخ‌های سلول‌های T را سرکوب می‌کند. علاوه بر این، گیرنده Toll-like receptor 9 (TLR-9) نیز در پاسخ‌های ایمنی ذاتی نقش دارد؛ این گیرنده DNA باکتریایی را شناسایی کرده و در ایجاد التهاب مزمن مشارکت می‌کند. هدف از این مطالعه، ارزیابی سطوح سرمی TLR-9، CD28 و CD152 در بیماران مبتلا به CSOM و بررسی ارتباط آن‌ها با الگوهای عفونت باکتریایی بود.

مواد و روش‌ها: در این مطالعه، ۵۰ بیمار مبتلا به CSOM و ۲۵ فرد سالم به‌عنوان گروه کنترل مورد بررسی قرار گرفتند. افرادی که بیش از سه ماه دچار ترشح مداوم گوش بودند و پارگی پرده صماخ آن‌ها با معاینات اتوسکوپیک و رادیولوژیک تأیید شده بود، به‌عنوان مبتلا به CSOM در نظر گرفته شدند. از بیماران نمونه سواب گوش جمع‌آوری و برای جداسازی باکتری‌ها کشت داده شد. شناسایی باکتری‌ها با استفاده از سیستم VITEK 2 Compact انجام گرفت. برای اندازه‌گیری سطوح سرمی TLR-9، CD28 و CD152 در نمونه‌های خونی تمام شرکت‌کنندگان، از روش الایزا (ELISA) استفاده شد.

نتایج: شایع‌ترین باکتری‌های جداشده در این مطالعه شامل *Staphylococcus aureus* (۳۸٪)، *Pseudomonas aeruginosa* (۲۶٪)، *Proteus mirabilis* (۱۴٪)، *Klebsiella pneumoniae* (۱۲٪)، و *Escherichia coli* (۱۰٪) بودند. سطح سرمی TLR-9 در بیماران مبتلا به CSOM به‌طور معنی‌داری بالاتر از گروه کنترل بود (۰/۰۷ ± ۱/۶۲ در برابر ۰/۰۵ ± ۰/۴۸ نانوگرم بر میلی‌لیتر؛ P < ۰/۰۰۱). سطح CD28 در بیماران به‌طور معنی‌داری کمتر از افراد سالم بود (۰/۰۹ ± ۰/۴۲ در برابر ۰/۰۶ ± ۱/۱۸ نانوگرم بر میلی‌لیتر؛ P < ۰/۰۰۱). در مقابل، سطح CD152 در بیماران CSOM به‌طور معنی‌داری افزایش یافته بود (۰/۱۱ ± ۱/۳۳ در برابر ۰/۰۸ ± ۰/۲۶ نانوگرم/میلی‌لیتر؛ P < ۰/۰۰۱). همچنین، همبستگی مثبت معنی‌داری بین عفونت *S. aureus* و بیان TLR-9 (r =۰/۶۹) و نیز بین عفونت *P. aeruginosa* و افزایش سطح CD152 (r =۰/۶۱) مشاهده شد.

نتیجه‌گیری: نتایج این مطالعه نشان داد که CSOM با افزایش بیان TLR-9 و CD152 و کاهش سطح CD28 همراه است. می‌توان نتیجه گرفت که این الگوی ایمنی ممکن است منجر به افزایش تحمل ایمنی، پایداری باکتری‌ها و تداوم التهاب مزمن در CSOM شود.

کلمات کلیدی: اوتیت میانی چرکی مزمن، تعدیل ایمنی، گیرنده Toll-like receptor 9، *Pseudomonas aeruginosa*، *Staphylococcus aureus*

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