ORIGINAL ARTICLE

Relationship of T lymphocytes, cytokines, immunoglobulin E and nitric oxide with otitis media with effusion in children and their clinical significances

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SUMMARY

OBJECTIVE: *To investigate the relations of T lymphocytes, cytokines, immunoglobulin E, and nitric oxide with otitis media with effusion (OME) in children and their clinical significances.*

METHODS: *Fifty children with OME treated in our hospital were enrolled in the study (observation group). Fifty healthy children were selected as control. The percentages of CD_4+ and CD_8+ T lymphocyte and CD_4+/CD_8+ ratio in peripheral blood, and the levels of cytokine (IL)-2, IL-4, IL-6, immunoglobulin E (IgE) and nitric oxide (NO) in peripheral blood and middle ear effusion (MEE) in both groups were detected. The correlations of these indexes with OME were analyzed.*

RESULTS: *The percentage of peripheral blood CD4+ and CD_8+ levels, CD_4+/CD_8 ratio, IgE, and NO levels in the observation group were significantly higher than those in the control group ($P < 0.01$). In the observation group, the IL-2 and IL-6 levels, and IgE and NO levels in the MEE were significantly higher than those in peripheral blood ($P < 0.01$). In addition, in the observation group, the MEE IL-2 and IL-6 levels were positively correlated with peripheral blood CD_4+/CD_8+ ratio, respectively $r = 0.366$, $P = 0.009$; $r = 0.334$, $P = 0.018$.*

CONCLUSIONS: *The levels of peripheral blood CD_4+ and CD_8+ lymphocytes and MEE IL-2, IL-6, IgE, and NO levels are increased in children with OME. These indexes have provided significant clues for the diagnosis of OME in children.*

KEYWORDS: *Otitis media with effusion. T-lymphocytes. Cytokines. Immunoglobulin E. Nitric oxide.*

INTRODUCTION

Otitis media with effusion (OME) is a common disease in otology and one of the main causes of hearing loss. About 50%-90% of preschool children suffer from OME, and patients with recurrent OME account for 30%-40% of total OME patients[1]. The protracted course of OME can easily influence the hearing of children, which seriously threatens their growth and intellectual development[2]. The etiology of OME is

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not yet fully elucidated. Although otology scientists have carried out long and extensive research on this, they have yet to reach a consensus. It is believed that OME is associated with a dysfunctional eustachian tube (including mechanical and non-mechanical obstruction of the eustachian tube), infection, immune response, among others[3]. With the progress of molecular biology and immunology, more and more scholars believe that the immune response is the main reason for the formation of middle ear effusion (MEE) and its retention[4]. MEE comes from the eustachian tube, tympanum, and mastoid air cells mucosa. Whether MEE is serous, mucous, or purulent, its pathological exudation, secretion, and absorption are involved in the process of OME[5]. It is known that OME is related to the inflammatory reaction and immune response of the body[6,7]. Cytokines such as interleukin (IL)-2, IL-4, IL-6, and nitric oxide (NO) are highly expressed in the MEE in adults[8,9]. In addition, there is an abnormity of T lymphocytes and immunoglobulin E (IgE) in patients with OME[10,11]. This further suggests that the immune response is closely related to OME, but the expression and significance of these indicators in children are rarely reported. This study investigated the relationships of T lymphocytes, cytokines, IgE, and NO with OME in children and their clinical significances. The objective was to provide a basis for the diagnosis and prognosis evaluation of OME in children.

METHODS

Subjects

Fifty OME children treated in our hospital were enrolled in this study. There were 26 males and 24 females. Their age was 2-15 years, with an average age of 7.3±3.1 years. They presented various degrees of hearing loss. The clinical manifestations were shouting, no attention, learning disability, need of a high volume when listening on the phone and watching television, among others. The diagnostic criteria of OME were as follows: i) the tympanic membrane protruded outward, or there were visible liquid plane and/or air bubbles; ii) the acoustic impedance test showed B-type or C-type curve; iii) the pure-tone audiometry result was abnormal. All the children were diagnosed with OME. They were treated with tympanic membrane incision and tube operation under general anesthesia. In addition, 50 healthy children were selected as control. There were 28 males and 22 females. Their age was 3-13 years, with an average age of 6.8±2.4 years. They had no history of otitis media or any related disease. There was no significant difference in age or gender between the two groups ($P > 0.05$). This study was approved by the ethics committee of the Shanghai Children's Hospital. Written informed consent was obtained from a family member of all participants.

Collection of specimens

Five milliliters of venous blood was taken from all subjects. Two milliliters of venous blood was placed in the EDTA anticoagulant tube (Sigma-Aldrich Corp., MO, USA) for measurement, and the remaining 3 ml was centrifuged at 2 000 r/min for 10 min. The serum was kept in a cryogenic refrigerator at -20 °C for measurement. In OME children, the cerumen in the external auditory canal was cleaned. The external auditory canal and its outer aperture were wiped with 75% alcohol cotton. The cotton contained 1% Dicaine (Jiangsu Jiuxu Pharmaceutical Co., Ltd., Xuzhou, China) and was gently attached to the ear tympanic membrane to relieve the pain of patients. After 15 min, the cotton piece was removed, and the external auditory canal and its outer aperture were disinfected with cotton and 75% alcohol (Jiangsu Jiuxu Pharmaceutical Co., Ltd.., Xuzhou, China) again. Under photopic vision (frontal mirror) or otoscope, a #5 needle was pierced into the tympanum from underneath the tympanic membrane. The MEM was extracted using 1 ml sterile syringe. The volume and mature of MEM were recorded. The MEM was placed in a sterile tube in the freezer at -20 °C for measurement.

Determination of indexes

The peripheral blood CD_4^+ and CD_8^+ levels were determined using flow cytometry[12]. The peripheral blood and MEM IL-2, IL-4, IL-6, and NO levels were determined using enzyme-linked immunosorbent assay[13]. The peripheral blood and MEM IgE levels were detected by chemiluminescence immunoassay[14]. The operations were followed the instructions of the kit's manufacturers (Sigma-Aldrich Corp., MO, USA).

Statistical analysis

All statistical analyses were carried out using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). The data were presented as mean ± SD. Comparisons between the two groups were performed using the t-test. The correlation of continuous variables was investigated

using the Pearson correlation analysis. $P < 0.05$ and $P < 0.01$ were considered statistically significant and highly statistically significant, respectively.

RESULTS

General data of OME children

In 50 OME children, the course of the disease varied from 5 days to 3 years, with an average of 4 months. There were 20 cases with less than 15 days of disease course and 30 with over 15 days. The otoscopy found tympanic membrane sign disappearance and light cone deformation. The acoustic impedance mapping showed 47 cases of B type (38 cases of both ears, 6 cases of right ear, 3 cases of left ear) and 3 cases of C type (1 case of both ears, 2 cases of right ear). The pure-tone audiometry showed a mild or moderate conductive hearing loss. The average language hearing loss was 35-55 dB, and the bone conduction difference was ≥ 30 dB.

Peripheral blood CD_4^+ and CD_8^+ levels in both groups

The peripheral blood CD_4^+ and CD_8^+ levels and CD_4^+/CD_8^+ ratio in the observation group were 12.27±3.67%, 7.78±2.12%, and 1.58±0.32, respectively, which were significantly higher than the 7.46±2.37%, 5.52±2.01%, and 1.35±0.19 found in the control group (all $P < 0.01$).

Peripheral blood and MEE IL-2, IL-4 and IL-6 levels in both groups

The peripheral blood IL-2, IL-4, and IL-6 levels in the observation group were 229.94±89.44 ng/L, 28.93±11.29 ng/L, and 33.18±10.41 ng/L, respectively, while in the control group they were 198.83±75.23 ng/L, 26.04±9.28 ng/L, and 29.88±6.23 ng/L. Each index in the observation group was higher than the same in the control group; however, the difference was not significant (all $P > 0.05$) (Table 1). In addition, in the observation group, the MEE IL-2, IL-4, and IL-6 levels were 705.19±147.37 ng/L, 35.25±23.31 ng/L, and 67.22±21.04 ng/L, respectively, while the peripheral blood IL-2, IL-4, and IL-6 levels were 229.94±89.44 ng/L, 28.93±11.29 ng/L, and 33.18±10.41 ng/L, respectively. In this group, the IL-2 and IL-6 levels in MEE were significantly higher than those in peripheral blood (all $P < 0.01$).

Peripheral blood and MEE IgE and NO levels in both groups

The peripheral blood IgE and NO levels in the observation group were 1245.36±458.46 mg/L and 74.56±23.72 ng/L, respectively, which were significantly higher than the 856.28±201.26 mg/L and 33.36±8.20 ng/L of the control group (all $P < 0.01$) (Table 2). In addition, in the observation group, the IgE and NO levels in MEE were 3205.28±660.39 mg/L

TABLE 1. COMPARISON OF PERIPHERAL BLOOD IL-2, IL-4, AND IL-6 LEVELS BETWEEN THE OBSERVATION AND CONTROL GROUPS

Group	IL-2 (ng/L)	IL-4 (ng/L)	IL-6 (ng/L)
Observation (n = 50)	229.94±89.44	28.93±11.29	33.18±10.41
Control (n = 50)	198.83±75.23	26.04±9.28	29.88±6.23
t	1.882	1.398	1.923
P	0.063	0.165	0.057

IL-2, interleukin-2; IL-4, interleukin-4; IL-6, interleukin-6.

TABLE 2. COMPARISON OF PERIPHERAL BLOOD IGE AND NO LEVELS BETWEEN THE OBSERVATION AND CONTROL GROUPS

Group	IgE (mg/L)	NO (ng/L)
Observation (n = 50)	1245.36±458.46	74.56±23.72
Control (n = 50)	856.28±201.26	33.36±8.20
t	5.495	11.608
P	< 0.001	< 0.001

IgE, immunoglobulin E; NO, nitric oxide.

and 423.44±67.67 ng/L, respectively, which were significantly higher than the 1245.36±458.46 mg/L and 74.56±23.72 ng/L in peripheral blood (all $P < 0.01$).

Results of the correlation analysis

Pearson correlation analysis showed that, in the 50 OME children, the MEE IL-2 and IL-6 levels were positively correlated with the peripheral blood CD_4^+/CD_8^+ ratio, respectively (IL-2 with CD_4^+/CD_8^+: $r = 0.366$, $P = 0.009$; IL-6 with CD_4^+/CD_8: $r = 0.334$, $P = 0.018$) (Figure 1). There was no significant correlation between the other two indexes ($P > 0.05$).

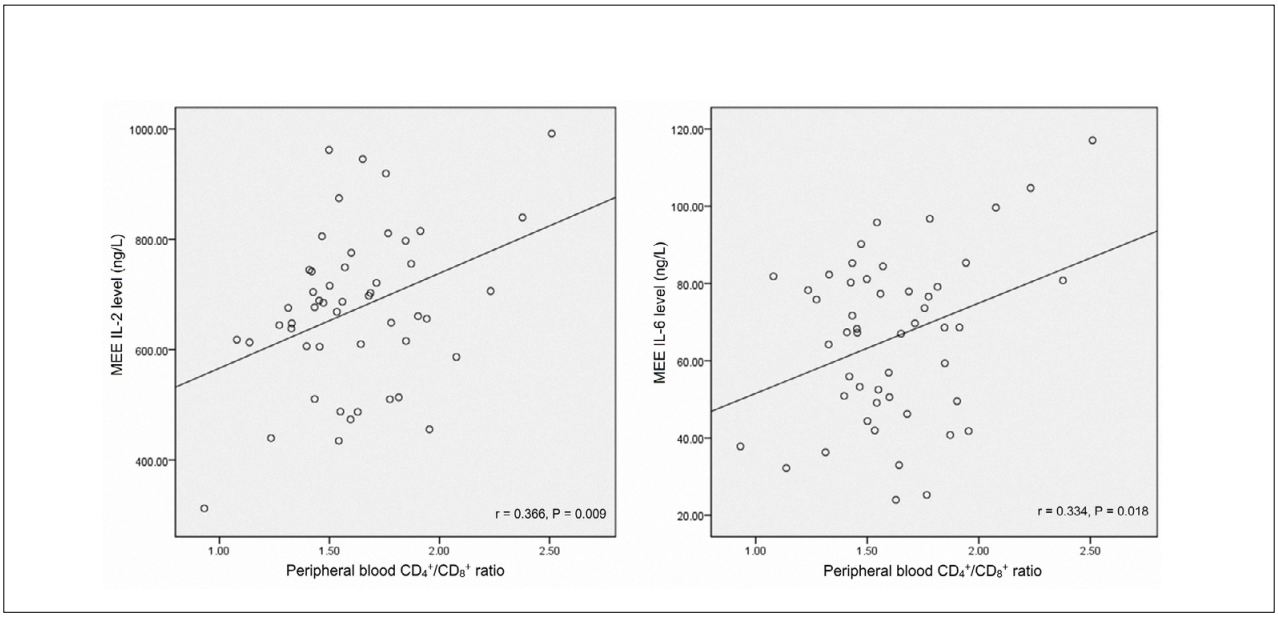
DISCUSSION

It is known that cytokines are cell regulating proteins with a variety of bioactivity that can mediate many immune responses in the body[15]. Generally, the basic functions of IL-2 and IL-4 are to activate immunity and stimulate the differentiation of B cells into plasma cells that produce immunoglobulin[16]. IL-6 is one of the essential factors that induce cell secretion and participates in the body defense reaction[17]. In this study, the peripheral blood IL-2, IL-4, and IL-6 levels in the observation group were higher than those in the control group, but the difference was not significant. In addition, in the observation group, the MEE IL-2 and IL-6 levels were significantly higher than the peripheral blood IL-2 and IL-6 levels. This indicates that in OME children IL-2 and IL-6 do not change in the peripheral blood, but are highly expressed in the MEE.

Hence, OME is related to the immune response of the body, and T lymphocytes are involved in the process of OME. CD_4^+ and CD_8^+ are an important part of the immunoregulation of T cells. CD_4^+ can help T cells produce antibodies, and CD_8^+ can inhibit the proliferation of T cells and the synthesis of antibodies. Both of them are directly or indirectly involved in the immune response[18]. Results of this study showed that the peripheral blood CD_4^+ and CD_8^+ levels and CD_4^+/CD_8^+ ratio in the observation group were significantly higher than those in the control group. This indicates that the inflammatory infection reaction occurs in children with OME, which stimulates the stress response of the body's immune function.

NO is an oxide produced by L-arginine catalyzed by nitric oxide synthase. It has an antimicrobial effect and is also an immunomodulator[19]. However, excessive NO often induces immune and pathological processes and mediates the production of some cytokines and inflammatory mediators, resulting in tissue damage[20]. Lin et al.[20] have reported that NO is highly expressed in the later stage of OME in adults. They speculate that the tissue damage and antibody inhibition caused by NO may be one of the critical factors leading to prolonged immobility of OME. In the present study, the peripheral blood NO level in the

FIGURE 1. CORRELATIONS BETWEEN MEE IL-2 LEVEL AND PERIPHERAL BLOOD CD_4+/CD_8+ RATIO AND BETWEEN MEE IL-6 LEVEL AND PERIPHERAL BLOOD CD_4+/CD_8+ RATIO. MEE, MIDDLE EAR EFFUSION; IL-2, INTERLEUKIN-2; IL-6, INTERLEUKIN-6.



observation group was significantly higher than that in the control group, and in the observation group, the MEE NO level was significantly higher than that in peripheral blood. This indicates that NO is involved in the process of OME in children.

Upper respiratory tract virus infection can cause IgE-mediated reaction, and IgE-mediated hypersensitivity is more likely to occur in patients with a family history of allergy. It often occurs in the nasopharynx, which can easily affect the eustachian tube[21]. In addition to the hypersensitivity reaction, the virus can reduce the function of granulocyte and lymphocyte, and significantly inhibit the cilium movement[22]. All these effects lead to eustachian tube obstruction and increased mucus secretion, as well as the accompanying dysfunction of ventilation and drainage. The results of this study showed that the peripheral blood IgE level in the observation group was significantly higher than that in the control group, while in the observation group, the MEE IgE level was significantly higher than that in peripheral blood. This indicates that IgE is involved in the process of OME in children. IgE mediated hypersensitivity exists in the peripheral blood and MEE in children with OME, which leads to the obstruction of the eustachian tube and increased mucus secretion.

There are certain correlations among T lymphocytes, cytokines, immunoglobulin E, and nitric oxide in the body. Liang et al.[23] found that in rats with moderate and severe traumatic brain damage the serum level of IL-2 is positively correlated with the serum level of CD_4^+/CD_8^+ ratio. Shu et al.[24] found that in early acute pancreatitis patients the serum level of IL-6 is positively correlated with the serum level of CD_4^+/CD_8^+ ratio. The results of Xu and Liu's study[25] have shown that in children with allergic rhinitis the serum IgE level is positively correlated with the CD_4^+/CD_8^+ ratio. In the present study, the correlation analysis showed that in 50 OME children the MEE IL-2 and IL-6 levels were positively correlated with peripheral blood CD_4^+/CD_8^+ ratio. This is consistent with the results previously found.

CONCLUSION

In conclusion, the levels of peripheral blood CD_4^+ and CD_8^+ T lymphocytes, and MEE IL-2, IL-6, IgE, and NO levels are increased in children with OME. These indexes have provided significant clues for the diagnosis of OME in children. This study had some limitations, its sample size is relatively small, which may affect the results. In subsequent studies, the sample size should be increased for more reliable results. In addition, there are many other indexes involved in the process of OME in children. This should be considered in further studies.

Disclosure of conflict of interest: None.

RESUMO

OBJETIVO: *Investigar as relações entre linfócitos T, citocinas, imunoglobulina E e óxido nítrico e a otite média com efusão (OME) em crianças e sua significância clínica.*

MÉTODOS: *Cinquenta crianças com OME tratadas em nosso hospital foram incluídas no estudo (grupo de observação). Selecionamos também 50 crianças saudáveis como controle. As porcentagens de linfócitos T CD_4+ e CD_8+ e a razão CD_4+/CD_8+ no sangue periférico, além dos níveis das citocinas IL-2, IL-4, IL-6, imunoglobulina E (IgE) e óxido nítrico (NO) no sangue periférico e de efusão no ouvido médio (MEE) de ambos os grupos foram medidos. A correlação desses índices com a OME foi analisada.*

RESULTADOS: *A porcentagem dos níveis de CD_4+ e CD_8+, da razão CD_4+/CD_8+, de IgE e NO no sangue periférico do grupo de observação foram significativamente maiores do que no grupo controle (P < 0,01). No grupo de observação, os níveis de IL-2 e IL-6, IgE e NO em MEE foram significativamente maiores do que no sangue periférico (P < 0,01). Além disso, no grupo de observação, foi encontrada uma correlação positiva entre os níveis de IL-2 e IL-6 em MEE e a razão de CD_4+/CD_8+no sangue periférico, respectivamente, r = 0,366, P = 0,009; r = 0,334, P = 0,018.*

CONCLUSÃO: *Os níveis de linfócitos CD_4+ e CD_8+ no sangue periférico e IL-2, IL-6, IgE e NO em MEE são mais altos em crianças com OME. Esses índices forneceram evidências valiosas para o diagnóstico de OME em crianças.*

PALAVRAS-CHAVE: *Otite média com derrame. Linfócitos T. Citocinas. Imunoglobulina E. Óxido nítrico.*

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