Objective: To isolate and identify the different bacterial pathogens causing acute otitis media (AOM), Chronic suppurative otitis media (CSOM) and secretory otitis media (OME). Also to evaluate the role of IL-8 in different type of otitis media (OM).

Materials and Method: Middle ear fluids were collected from 103 patients suffering from different types of OM. Patients separated into 3 groups: group I (AOM), group II (CSOM) and group III (OME). Middle ear fluid was collected and subjected to bacteriological study and assessment of IL-8.

Results: Positive cultures were detected in 68.7% in group I, 88.1% in group II while no bacterial growth was detected in group III. IL-8 was detected in the 3 groups with statistical significance between the 3 groups, it was evident between group I and III and between group II and III. There was significant correlation between the results of bacterial culture and the level of IL-8.

Conclusions: IL-8 plays a role in the development of chronicity of OM. It has intimate relation to the bacterial growth; it acts as a chemo-attractant to neutrophils into the middle ear fluid.

Introduction

Otitis media results from a complex interaction of both cellular and humoral immune and inflammatory mediators. The most significant elements in the pathogenesis of the inflammatory process are Eustachian tube dysfunction and infection leading to accumulation of inflammatory cells along with cytokines, immunoglobulin and other mediators in the middle ears[1].

Acute otitis media (AOM) is one of the most common infectious diseases in childhood. In a survey of the frequency of infectious diseases during the first year of life only the common cold was more common[2]. Over 90% of children will experience at least one episode in their lives, while over 70% of children will have 3 or more episodes. Most of these episodes occur within the first two years of life, 50% of first episodes occurring by the third year of life[3].

Otitis media with effusion (OME) is one of the most prevalent middle ear diseases, characterized by retention of effusion in the middle ear cavity[4]. The pathogenesis of OME involves chronic accumulation of inflammatory (especially neutrophils) and immune effectors cells in the middle ear cavity[5].

Recently, a variety of cytokines have been demonstrated in the middle ear effusions. These cytokines include IL-1β, IL-2, IL-6, tumor necrosis factor, interferon and IL-8[6-7].

Retention of cytokines in the middle ear cleft during persistent OME may results in an ongoing inflammatory state that has the potential for mucosal changes, fibrosis, bone erosion and hearing loss[8].

The inflammatory cytokine IL-8 plays a crucial role in the initiation and maintenance of the inflammation process in variety of tissues. It functions as a potent neutrophilic chemo-attractant. IL-8 is present in the middle ear effusionsof children andadults and is though to be responsible for the accumulation and activation of neutrophils in the middle ear effusions[9].

Russo et al[10], reported that IL-8 plays a central role in the chronicity of middle ear effusion, they reported that this cytokine is present in both AOM and OME.

This work was done in attempt to isolate and identify different bacterial pathogens causing AOM, chronic suppurative OM (CSOM), and OME. In addition, to evaluate the role of interleukin-8 in different types of otitis media, especially its role as chemokine to neutrophils and in induction of chronicity and tissue damage in middle ear.
Patients and methods
This study was conducted on 103 patients suffering from different types of otitis media at Otorhinolaryngology and Microbiology Departments, Mansoura University from April 2003 to April 2007. Patients were classified according to the nature of the disease into 3 groups. Group I included patients suffering from AOM (16 patients), group II included patients suffering from CSOM (42 patients) and group III included patients suffering from OME (45 patients). Middle ear fluids were collected and sent to the microbiology Department for immunological and bacteriological study. In cases with AOM with bulging drum, the samples were collected at the time of myringotomy, in cases with OME, the samples were collected at the time of ventilation tube insertion while in cases with CSOM and AOM after perforation, samples were collected by aspiration through the tympanic membrane perforation.

Method for samples collection
Middle ear fluids were collected from the patients of the studied groups in a sterile syringe by tympanocentesis following the method described by Brook et al. [11]. The external auditory canal was filled with povidone-iodine solution for 3 minutes. Removal of this solution was accomplished by irrigation with 50 ml of sterile saline solution, and the excess saline solution was absorbed with sterile cotton. With a surgical microscope, incision was made in the tympanic membrane. Under direct visualization, the effusion was aspirated through sterile plastic syringe, which was connected to suitable suction apparatus.

Middle ear fluids were transported immediately to laboratory in Microbiology Diagnostic Infection Control Unit (MDICU) in Medical Microbiology and Immunology Department. Specimens were plated within 5 to 10 minutes of collection. All samples are subjected to bacteriological and immunological study. The Immunological study included Human IL-8 immunoassay, total leucocyte count and differential leucocyte count for neutrophil percent.

Microbiological examination
Samples taken from the patients were examined for bacteria using stranded microbiological techniques. Direct smear stained by gram stain, culture for 24-48 hours at 37°C at blood agar, chocolate agar, McConkey’s agar and nutrient agar.

Immunological study
Equal volumes of MEE and sterile saline were centrifuged at 1200 rpm for 10 min. the supernatant was preserved at -20°C for IL8 estimation, while the pellet was separated and subjected for total leucocytic count and differential count to estimate neutrophil percent and number.

Statistical analysis was carried out through SPSS program version [10]. The quantitative data were presented in the form of mean and standard deviation. Student T test was done to compare between each two groups. Chi-square was used for qualitative data. Results were considered significant when P value is < 0.05.

Results
There were 16 patients in group I (9 male and 7 female), 42 patients in group II (22 male and 20 female) and 45 patients in group III (23 male and 22 female). The distribution of age, sex, side and duration of the disease are demonstrated in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of patients</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Side of disease</th>
<th>Duration of the disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>16 (15.5%)</td>
<td>14.35 (0.7-40)</td>
<td>9 (56.3%)</td>
<td>7 (43.7%)</td>
<td>7 (43.7%)</td>
</tr>
<tr>
<td>Group II</td>
<td>42 (40.8%)</td>
<td>15.29 (6-32)</td>
<td>22 (52.4%)</td>
<td>20 (47.6%)</td>
<td>22 (52.4%)</td>
</tr>
<tr>
<td>Group III</td>
<td>45 (43.7%)</td>
<td>18.93 (6-36)</td>
<td>23 (51.1%)</td>
<td>22 (35.6%)</td>
<td>23 (51.1%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Culture positive cases</th>
<th>Culture negative cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I  (11 (68.7%))</td>
<td>5 (31.3%)</td>
</tr>
<tr>
<td>Group II (37 (88.1%))</td>
<td>5 (11.9%)</td>
</tr>
<tr>
<td>Group III (0)</td>
<td>45 (100%)</td>
</tr>
</tbody>
</table>

Table 1. Number and per cent of patients in the three studied groups in relation to age, sex, side of disease, and duration of the disease.

Table 2. Number and per cent of culture positive cases and culture negative cases in the study groups.
group I and 37 cases (88.1%) in group II while there was no bacterial growth in cultures of group III. Positive and negative cultures are demonstrated in Table 2.

The commonest organisms isolated from group I were β haemolytic Streptococci and Haemophilus species (18.8% for each), followed by Streptococcal pneumoniae (12.5%), while in group II the commonest organisms isolated were Pseudomonas aeruginosa (21.4%), followed by Proteus species (21.4%). The distribution of isolated bacteria is demonstrated in Table 3.

The level of IL-8 (ng/ml) was ranging from 100 to 154 with a mean value of 128 ± 18.69 in group I, from 110 to 185 with a mean value of 158.36 ± 38.52 in group II and from 41 to 71 with a mean value of 55.84 ± 10.73 in group III. There was a statistical significant difference in the level of IL-8 among the study groups, it was more evident between group I and III and between group II and III P<0.05.

We studied also the levels of IL-8 in relation to the age in the three groups; there were no significant relations between the IL-8 level and the age of the patients.

The levels of IL-8 in relation to bacterial growth were also evaluated; there was a significant correlation with both positive and negative bacterial cultures (P<0.001).

The percent and count (x10³) of neutrophils were detected in the study groups; it ranged from 65 to 84 with a mean value of 75.8±11.52 (percent), and from 15.7 to 42.1 with a mean value of 26.99±1.7 (count) in group I, ranged from 70 to 90 with a mean value of 82.5±5.13 (percent), and from 19.4 to 47.2 with a mean value of 40.3±1.9 (count) in group II and ranged from 50 to 70 with a mean value of 57.42±5.06 (percent), and from 2.3 to 4.5 with a mean value of 3.38±0.5 (count) in group III. There was a statistical significant difference among the three studied groups regarding the percent and count (P value < 0.005).

Neutrophil count and percent in total positive growth were higher than total negative growth.

There was a significant correlation between IL-8 and neutrophil percent and count in middle ear fluid in the three studied groups (P value < 0.005).

Discussion

Our data showed that, a positive bacterial growth was detected in 68.75% of cases in group I, 88.1% of cases in group II and no bacterial growth was detected in any case of group III, suggesting that the middle ear effusion in this group is sterile.

These findings are in agreement with Li et al[12] who reported 70%-78% positive culture in AOM and in agreement with Kuczkowski et al[13] who reported 81%-87% positive culture in CSOM.

Some of our cases (in group I and II) did not give any bacterial growth, the same findings were observed by Prellner et al[14] and this may be attributed to viral etiology, the prior administration of antibiotics or suppression of the growth by the immunological response of the host.

Also our data regarding group III are in agreement with that reported by Hurst and Venge[15] who stated that, 91% of OME patients were atopic and this may explain the sterile nature of the fluid in these cases.

Table 3. The distribution of different isolated bacteria in the study groups.

<table>
<thead>
<tr>
<th>Isolated bacteria</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>β haemolytic strept.</td>
<td>3 (18.8%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Candida species</td>
<td>0</td>
<td>1 (2.4%)</td>
<td>0</td>
</tr>
<tr>
<td>Citrobacter species</td>
<td>0</td>
<td>6 (14.3%)</td>
<td>0</td>
</tr>
<tr>
<td>E.coli</td>
<td>1 (6.3%)</td>
<td>4 (9.5%)</td>
<td>0</td>
</tr>
<tr>
<td>Haemophilus species</td>
<td>3 (18.8%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Klebsiella species</td>
<td>0</td>
<td>4 (9.5%)</td>
<td>0</td>
</tr>
<tr>
<td>MRSA</td>
<td>0</td>
<td>1 (2.4%)</td>
<td>0</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>0</td>
<td>4 (9.5%)</td>
<td>0</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>0</td>
<td>5 (11.9%)</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>0</td>
<td>9 (21.4%)</td>
<td>0</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>1 (6.3%)</td>
<td>3 (7.1%)</td>
<td>0</td>
</tr>
<tr>
<td>Staph. epidermidis</td>
<td>1 (6.3%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Strept. pneumonia</td>
<td>2 (12.5%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Our data showed also that, the most common bacterial isolates in group I were Haemophilus species and β hemolytic streptococci (18.8% for each) followed by Strept. pneumoniae (12.5%), and lastly Staph. aureus, Staph. epidermidis and E. coli (6.3% for each). These findings are in agreement with that reported by Li et al[12].

In agreement with Kuczkowski et al[13], our results showed that the most common isolated bacteria in group II were Pseudomonas aeruginosa and Proteus species.

IL-8 is secreted by a number of inflammatory cell types, including neutrophils, T-cells, monocytes/macrophages, eosinophils and natural killer cells [16, 17].

Our data demonstrate significant higher level of IL-8 in group I and II. Similar results were reported by Russo et al[10].

These higher levels may emphasize the role of IL-8 in development of chronicity and its intimate relation to bacterial growth and thus explains the low level of IL-8 in group III.

Our data clarifies that, the difference in neutrophil percent and count among the three studied groups is parallel to the difference in level of IL-8. Our findings are in agreement with that reported by Nassif et al. [9]. This finding supports the hypothesis that IL-8 recruits neutrophils to the middle fluids.

The correlation between IL-8 and neutrophil percent in our work could be attributed to the fact that, IL-8 is a potent neutrophil chemoattractant and activator[10].

Our results suggested that IL-8 plays an important role in the pathogenesis of OM as follow:

• IL-8 enhances inflammations by promoting the degranulation of neutrophil-specific granules and adherence of neutrophil to the endothelial cells and subendothelial matrix proteins[19].
• IL-8 accelerates the recruitment of neutrophils into the middle ear cavity and increases the release of lysosomal enzymes; IL-8 plays a key role in causing tissue damage, which leads to the prolongation of ME inflammation[20].
• IL-8 plays a central role in the chronicity and duration of MEE[10].

The neutrophil chemotactic and activating properties of this cytokine may be responsible for mucosal damage and thus the chronicity of the disease[21].

Conclusion

- the commonest organisms isolated in AOM were β haemolytic Streptococci and Haemophilus species, while in CSOM were Pseudomonas aeruginosa followed by Proteus species. There was no bacterial growth in all cases with OME.
- IL-8 plays a role in development of chronicity and it has intimate relation to the bacterial growth.
- IL-8 acts as chemoattractant to the neutrophils into the middle ear cavity.
- The neutrophil chemotactic and activating properties of this cytokine may be responsible for mucosal damage and thus the chronicity of the disease.

References

The Role of IL-8 in Different Types Of Otitis Media And Bacteriological Correlation


