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Evaluation of primary cytokines and lactate dehydrogenase from nasopharyngeal secretion of Acute Otitis Media (AOM)

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ABSTRACT

Background: Acute otitis media (AOM) is a common side effect of a viral upper respiratory tract infection (URI). The severity of nasopharyngeal cellular injury during URI, as evaluated by lactate dehydrogenase (LDH) concentrations in nasopharyngeal secretions (NPSs), was hypothesized to be associated with AOM complications.

Methodology: LDH concentrations were measured in NPS samples (n = 110) obtained during the initial URI followed by the development of AOM. Interleukin-6 (IL-6), and tumor necrosis factor- α (TNF-α)- concentrations were also measured. Lactate Dehydrogenase (LDH) and cytokines were measured by Immunoassay methods. The ELISA-based immunoassay Limit of Detection (LOD) for LDH is 0.1-1.0 mU/ml and the conventional Enzyme-Linked Immunosorbent assay (ELISA) LOD for IL-6 and TNF-α is from 0.5 to 5.0 pg/ml and 0–15 pg/ml, respectively.

Results: LDH concentrations independently predicted AOM complications. LDH concentrations were higher in URIs ($P \leq 0.05$). There was a positive correlation ($P<0.001$) between LDH concentrations and all cytokines.

Conclusion: LDH concentrations in NPS are related to an increased risk of AOM, implying that the severity of nasopharyngeal inflammatory injury during URI contributes to the development of AOM and that reducing inflammatory injury may lessen the risk of AOM.

Keywords: *Acute otitis Media; Nasopharyngeal secretions; Lactate dehydrogenase; Cytokines.*

1. INTRODUCTION

Acute otitis media (AOM) is an infection of air-filled space behind the eardrum. It is one of the most common bacterial infections caused by otopathogens. AOM mostly occurs during and after viral upper respiratory tract infection and is highly prevalent among young children.[1] The pathogenesis of AOM is complex and involves interactions among the host, pathogen, and environmental factors. Usually, there is a preexisting nasopharyngeal colonization with pathogenic bacteria. Viral URI causes inflammation of the nasopharynx and eustachian tubes, which is mediated by substances such as cytokines and inflammatory mediators. [2] The inflammation leads to eustachian tube dysfunction, which, in turn, causes a negative

pressure in the middle ear, allowing the pathogens from the nasopharynx to enter the middle ear. This results in fluid or pus accumulation in the middle ear space, thereby causing AOM. The AOM complication occurs only in about one-third of young children. More severe nasopharyngeal inflammatory injury likely leads to a higher risk for AOM. [3] The risk factors for AOM are mainly host and environmental origin. The first group i.e. host related factors includes age, sex, ethnicity, family history of AOM and genetic predisposition, craniofacial anomalies, atopy, immunodeficiency, adenoid hypertrophy, and gastroesophageal reflux. The second group, environmental factors, include daycare attendance, passive smoking, older siblings, and use of pacifiers, etc. [4,5,6] Lactate dehydrogenase (LDH) is a membrane-associated enzyme and is released in the extracellular environment during inflammation as a result of cellular injury. LDH is an enzyme released in the bronchoalveolar space on damage to the cytoplasmic cell membrane. Elevated LDH is also an indicator of underlying lung injury and inflammation. The level of LDH is raised in any nasopharyngeal, bronchial injury, and in the middle ear effusions of patients with otitis media due to any source, e.g., malignancy, viral pneumonia, viral URI, or bacterial or mycoplasma infection, etc. [6,7] Cytokines are bioactive proteins that widely mediate host response to inflammatory stimuli. In the pathophysiology of respiratory infection, they regulate proliferation, chemotaxis, and the activation of inflammatory response. It is released from the numerous cell types found in the middle ear cleft such as epithelial cells, endothelial cells & various immune cells. [8] A host of inflammatory mediators are produced by tissue macrophages in response to microbes and their products, including prostaglandin E_2 (PGE_2), which causes vasodilatation and TNF-α, interleukin-1 (IL-1) and interleukin-8 (IL-8), which attract and guide neutrophils into the middle ear cavity. [9] Locally produced cytokines are considered to play an important role in the initiation and maintenance of inflammation. The major three pro-inflammatory cytokines are TNF-α, IL-1, IL-6, and anti-inflammatory cytokines, interleukin-1 receptor antagonist (IL-1Ra) and interleukin-10 (IL-10) down-regulate the inflammatory process. An anti-inflammatory activity has been observed in a significant percentage of exudates in patients with otitis media. [10] However, there has been previously separate published work on the use of LDH & cytokines concentrations in acute otitis media as a biomarker of inflammatory cellular injury during viral URI and the associated risk for AOM complication [9,10]. So, the present study has planned to determine the levels of LDH and primary cytokines levels (i.e., TNF-α & IL-6) and their relation in the development of AOM from the NPS sample.

2. METHODS

Between November 2022 and December 2023, patients who visited the ambulatory care centre at G B Pant Hospital, Andaman Nicobar Island with suggestive symptoms of AOM were initially screened for eligibility by the investigators. After obtaining the approval from the institutional ethical committee a total of 110 patients with age groups of 2-10 years of either gender were enrolled. Patients who had at least minimum one sign of inflammation i.e., ear pain, fever (>100.1°F or 37.8°C), redness and bulging of tympanic membrane and effusion in the middle ear after obtaining informed consent from the parents or guardians were enrolled for the current study. Patients with recurrent or history suggestive of chronic AOM were excluded from the study. For the investigation and analysis middle ear fluid was collected by tympanocentesis and immediately inoculated into brain heart infusion broth and sent for culture. Nasopharyngeal secretions (NPSs) have been collected at each initial visit for upper respiratory infection (URI) and AOM. Both have been tested for viral presence. The present study has only analysed the LDH and cytokines. The present study only included the early sample collection (i.e. within 7 days of URI onset). NPS sample was collected during subsequent visits and analysed for LDH and cytokines. 1 ml of sterile phosphate buffer saline (PBS) was used to rinse the suction tube. The volume of secretion in phosphate-buffered saline was measured and recorded. The original sample's dilution factor was calculated from the total volume (1 ml). Aliquots of NPSs were stored at −70°C until used for levels of LDH, TNF-α and IL-6. After that patients were instructed to follow the treatment procedure with a prescription of clarithromycin, 15 mg/kg/day. Total LDH activity was measured by using enzymatic methods from ERBA kit literature in the NPS samples was determined using a commercial ELISA immunoassay kit. The LDH concentration range was between 0.1-1.0 mU/ml

3. STATISTICAL ANALYSIS

Data of general characteristics were summarized in frequency form whereas quantitative data were represented in the form of mean & standard deviation (SD). The test of significance was calculated by using a standard t-test. Pearson correlation analysis was used to find the relationship between LDH and cytokines. SPSS 21 software was used for a detailed analysis of data collected from different sources.

4. RESULT

A total of 110 children with an age group of 2-10 years of which 80 were male and 30 were female represented in Figure 1. LDH and cytokine concentrations are represented in Table 1. Table 1 also highlights a relationship between nasopharyngeal secretions LDH to cytokines. A positive association was found between LDH and cytokines levels. To find out significant relationship between LDH and cytokines by using Pearson's correlation.

Figure 1: Gender-wise distribution of AOM Subjects

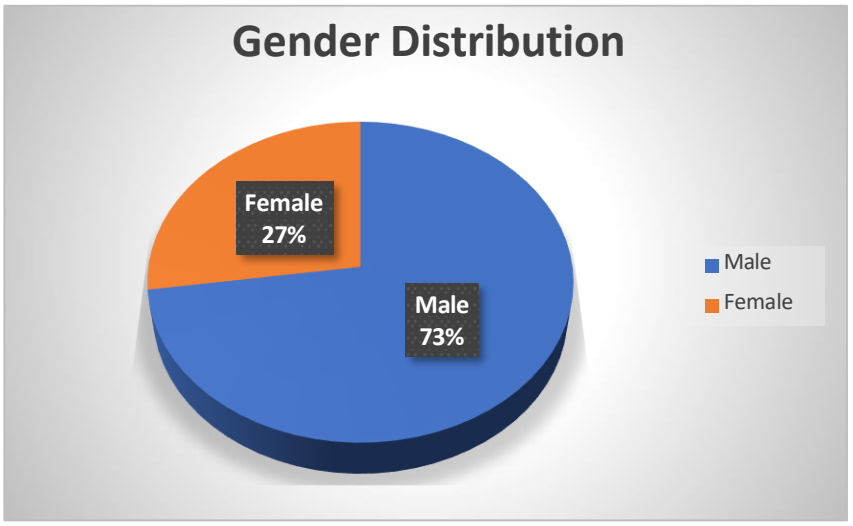

Table 1. TNF-α and IL-6 Concentration

Parameters		Statistical values
Age (Mean ± SD)		06 ± 02 years
LDH (Mean ± SD)		6.23 ± 3.25 mU/ml
IL-6 (Mean ± SD)		7.90 ± 2.57 pg/ml
TNF-α (Mean ± SD)		19.25 ± 2.30 pg/ml
Correlation with LDH levels	LDH with IL-6	r = 0.29 (P < 0.001)
	LDH with TNF-α	r = 0.34 (P < 0.001)

Table 1 presents the average age of study participants, along with the mean levels of LDH, IL-6, and TNF-α. It also highlights a significant positive Pearson correlation (r) between LDH and both IL-6 and TNF-α.

5. DISCUSSION

Otitis media has a multifactorial background. The pathogenesis of AOM is regulated by many internal and external factors including normal flora and individual immunological response. In this study, we studied children aged 2-10 years of child having nasopharyngeal inflammatory cellular injury as determined by the LDH levels from NPS samples which is significantly associated with the development of different AOM complications. Numerous studies have shown that the step-wise increase in AOM rates with increasing LDH levels. [11] The mechanism of nasopharyngeal tissue injury in URI leading to AOM may be complex, we postulate that severe nasopharyngeal tissue injury leads to AOM through eustachian tube dysfunction. LDH found in our NPS samples likely came from nasopharyngeal tissues because Schorn et al. [12] have shown that the serum compartment does not contribute significantly. The extracellular LDH itself has no known biological activity and is therefore simply a biomarker of cellular injury. Schorn et al. have also shown that LDH concentrations in NPS are higher during viral URI than during bacterial, allergic, or atrophic rhinitis. Several factors, including direct virus-induced cytopathic injury of the infected cells and leukocyte participation in both antibody-dependent and antibody-independent cytotoxicity (e.g., neutrophils, macrophages, and lymphocytes), contribute to nasopharyngeal injury during viral URI.[13] Various chemokines and cytokines, particularly acute phase cytokines like TNF-α and IL-1β, as well as other soluble mediators, can interact with the endothelium and epithelium of the surrounding tissues to promote leukocyte migration toward infected epithelial cells. These cells then contribute to the cytotoxic damage of neighbouring bystander cells and infected cells. [2]

The present study has shown a positive association of TNF-α and IL-6 with LDH concentration indicating that LDH is a reliable biomarker of acute inflammatory injury associated with URI. These cytokines, however, do not, by themselves, explicitly indicate cellular injury as does LDH; rather, acute-phase cytokines act as mediators of inflammation, while LDH

is the result of inflammatory injury. Nasopharyngeal epithelial cells are exposed to resident colonizing bacteria that may be pathogenic and induce AOM during viral URI. The contribution of bacteria to nasopharyngeal cellular damage during viral URI was not assessed in this investigation. Although there is no evidence that the intact, healthy nasopharyngeal epithelium may sustain cellular damage due to nasopharyngeal bacteria alone, their involvement in enhancing the inflammation caused by viral coinfection is well established, particularly through their interaction with leukocytes.[2] Our results showing a direct correlation between LDH and AOM are in contrast to those of Laham et al. [14] and Mansbach et al. [15], who showed a reduced severity of bronchiolitis, a lower airway complication due to viral infection, with high LDH concentrations in NPSs. The theory put forth by these researchers is that a strong immunological and inflammatory response in the upper airway, as evidenced by increased LDH in NPSs, guards against a more serious illness in the far-off lower airway. However, we suggest that an increased inflammatory response in the upper airway causes local difficulties such as eustachian tube dysfunction, which in turn causes AOM. [15]

The limitations of our study include its post-hoc analytical design and the absence of serial nasopharyngeal secretion (NPS) collections from the same child throughout upper respiratory infection (URI). Prospective studies are needed to validate our findings in diverse populations and to better define threshold LDH concentrations that predict acute otitis media (AOM) risk [16]. Furthermore, our results underscore the need for clinical trials evaluating topical anti-inflammatory treatments aimed at reducing nasopharyngeal mucosal injury in otitis-prone children, intending to prevent AOM complications.

6. CONCLUSION

- The severity of nasopharyngeal inflammatory injury during viral URI, as measured by LDH concentrations in NPSs, is a key determinant in the development of AOM.
- A strong positive correlation between cytokine levels and LDH may serve as a powerful predictor of disease severity.

LDH concentrations in NPS are related to an increased risk of AOM, implying that the severity of nasopharyngeal inflammatory injury during URI contributes to the development of AOM and that reducing inflammatory injury may lessen the risk of AOM.

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