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EVALUATION OF PRIMARY CYTOKINES AND LACTATE DEHYDROGENASE FROM NASOPHARYNGEAL SECRETIONS OF ACUTE OTITIS MEDIA

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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 to AOM complication**.**

Methodology: LDH concentrations were measured in NPS samples (n = 110) obtained during the initial URI followed by the development of AOM. Interleukin IL-6, and tumor necrosis factor (TNF)-concentrations were also measured. LDH and Cytokines were measured by Immunoassay methods. The LDH concentration minimal range was 0.9 and 1,000 mU/ml and the lowest cytokines detection limit of the assay was <10 pg/ml for IL-6 and alpha TNF.

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

Conclusion: LDH concentrations in NPS are related with 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.

Key words: Acute otitis Media; Nasopharyngeal secretions; Lactate dehydrogenase; cytokines.

Introduction:

Acute otitis media (AOM) is an infection of air-filled space behind the eardrum. A most common bacterial infection caused by otopathogens. AOM mostly occur 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 middle ear space, thereby causing AOM. The AOM complication occurs only in about one-third of young children. It is likely that more severe nasopharyngeal inflammatory injury leads to a higher risk for AOM. [3]

An AOM risk factors are mainly host and environmental origin. The first group i.e. host includes age, sex, ethnicity, family history of AOM and genetic predisposition, craniofacial anomalies, atopy, immunodeficiency, adenoid hypertrophy, gastroesophageal reflux. Second is environmental factors include day-care attendance, passive smoking, older siblings, and use of pacifier etc [4,5,6] Lactate dehydrogenase (LDH) is a membrane associated enzyme and is released in extracellular environment during inflammation as a result of cellular injury. LDH is enzyme released in the bronchoalveolar space on damage of cytoplasmic cell membrane. Elevated LDH is also 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, i.e., 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 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 tumour necrosis factor (TNF), interleukin-1 (IL-1) and IL-8, which attract and guide neutrophils into the middle ear cavity. [9]

Locally produced cytokines are considered to play an important role in initiation and maintenance of inflammation. The major three pro-inflammatory cytokines are tumor necrosis factor (TNF) α, interleukin-1 (IL-1), IL-6 and anti-inflammatory cytokines IL-1Ra and IL-10 down regulate the inflammatory process. An anti-inflammatory activity has been observed in 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 inflammatory cellular injury during viral URI and the associated risk for AOM complication. So, the present study has planned to determine the levels of LDH and primary cytokines levels (i.e., alpha TNF & IL-6) and their relation in development of AOM from NPS sample.

Methodology:

From the last one year (November-2022 to December-2023), patients came at ambulatory care center with complained of AOM symptoms were screened firstly by investigators for eligibility. After obtaining the approval from institutional ethical committee a total of 110 patients with age group of 2-10 years of either gender were enrolled. Patients 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 with received consent by parents and relatives were enrolled for current study. Patient with recurrent or suggestive chronic history of 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 virus lodge. The present study has only analysed the LDH and cytokines. Present study only included the early sample collection (i.e. within 7 days of URI onset). NPS sample were collected in subsequent visit and analysed for LDH and cytokines. 1 ml of sterile buffer saline was used to rinse suction tube. The volume of secretion in phosphate-buffered saline was measured and recorded. Dilution factor of the original sample was calculated from the total volume (1 ml). Aliquots of NPSs were stored at −70°C until used for levels of Lactate dehydrogenase (LDH), alpha tumor necrosis

factor (α-TNF) and interleukin-6 (IL-6). After that patients were instructed to follow treatment procedure with prescription of clarithromycin, 15 mg/kg/day.
Total LDH activity in the NPS samples was determined using a commercial immunoassay kit. The LDH concentration range was between 0.9 and 1,000 mU/ml. A subset of virus-positive NPS samples was analysed for IL-6, and TNF-α concentrations.

Statistical analysis:
Data of general characteristic were represented in frequency form whereas quantitative data were represented in the form mean & SD. Test of significance were calculated by using standard t test to find data significancy. Pearson correlation analysis was used to find relationship between LDH and cytokines.

Results:
A total 110 child with age group of 2-10 years of which 80 were male and 30 were female represented in figure-1. LDH and Cytokines concentration were represented in table-2. Table-2 also represented a relationship between NPS LDH to cytokines. A positive association were found between LDH and cytokines levels. P value was calculated by Pearson correlation test using natural log transformation of the LDH and cytokine concentration values.

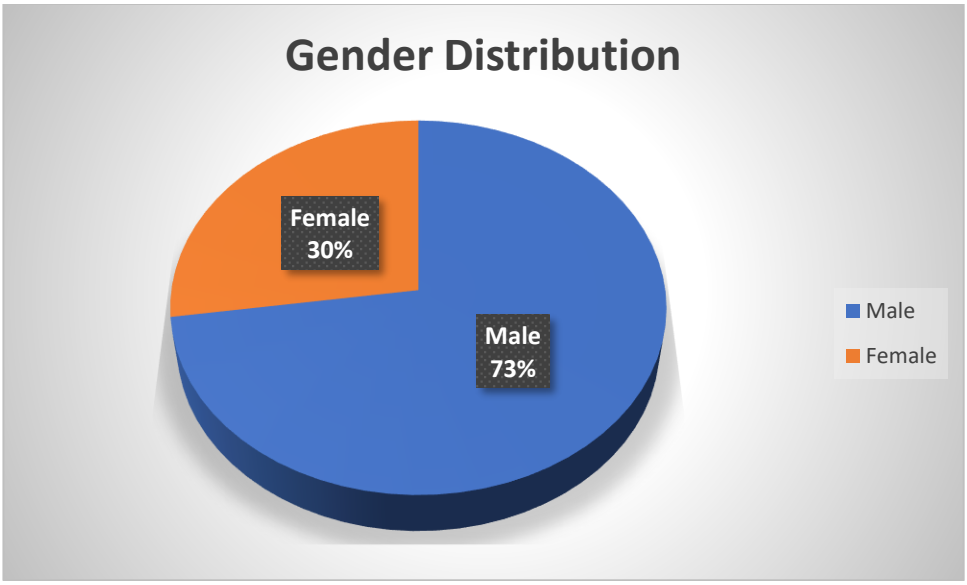

Figure-1: Gender wise distribution of AOM Subjects

Table-2: Statistical analysis of LDH and cytokines in AOM subjects.

Parameters		**Statistical values**
Age (Mean ± SD)		06 ± 02 years
LDH (Mean ± SD)		3,963 ± 3, 200 mU/ml
IL-6 (Mean ± SD)		7,907 ± 25,723 pg/ml
TNF-α (Mean ± SD)		3,169 ± 2, 304 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)

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 have taken the cases of 2-10 years of child having nasopharyngeal inflammatory cellular injury as determined with the LDH levels from NPS samples and which is significantly associated with development of different AOM complication. Numerous studies have shown that the step wise

increase in AOM rates with increasing LDH levels. Subsequently, the levels of LDH were increases day by day of onset of infection and it has shown significant association on fourth day of onset of infection in upper respiratory infection. [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 biologic 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. Number of 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), can lead to nasopharyngeal cellular 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. [14]

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 end 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.[14] Our results showing a direct correlation between LDH and AOM are in contrast to those of Laham et al. [15] and Mansbach et al. [16], 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 eustachian tube dysfunction, which in turn causes AOM. [16]

The limitations of our study include the post-hoc nature of data analysis and the lack of daily evaluations and NPS collections in the same child during the URI period. More prospective studies are needed to corroborate our findings in other groups and to identify the particular LDH concentrations at which AOM complications can be predicted more accurately. [17] Our findings further highlight the necessity for treatment trials of topical pharmacological medications that lower nasal cavity inflammatory injury in otitis-prone children to minimize the risk of AOM.

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.
- 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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