Secretory Immunoglobulin A In Suppurative Otitis Media

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Abstract

Background: Secretory Ig A constitute a local immunological factor protecting the middle ear against invasion of pathogens and prevention of tissue damage by toxins. The aim of this study was to determine the serum and middle ear secretion(MES) level of Ig A in acute(ASOM) and chronic suppurative otitis media (CSOM) and evaluate the possibility of using Ig A as index of chronicity.

Method: Enzyme immunodiffusion technique.

Result: The subjects and control were made of 30 males and 22 females, between 6 months and 9 years, mean of 6 years(SD =3.26).
Paired sera and middle ear secretion (MES) from 37 subjects with suppurative otitis media comprising of 20 CSOM and 17 ASOM and sera of 15 control were analysed. The mean serum levels of Ig A were control group 36mg/dL, ASOM 46.1mg/dL, CSOM 41mg/dL, while the MES levels were ASOM 228.3mg/dL and CSOM 85.4mg/dL. (Table 1).
The mean MES : serum ratio were 4.95 for ASOM and 2.1 for CSOM.
The ratio of the serum level of control group to ASOM was 1.3 while control to CSOM was 1.14.
Multivariate analysis using unpaired t-test to compare the mean revealed significant statistical difference between the MES level of ASOM and CSOM (P= 0.0056) but no significant difference between serum level of ASOM and CSOM(P=0.57), and serum level in control and ASOM (P=0.25).

Conclusion: The Ig A level in middle ear secretion was higher in ASOM than CSOM, thus reduced secretion may be associated with chronicity. The monitoring Ig A assay may be useful index of determining likely progression to chronicity, however, further study is still needed to establish this hypothesis.

INTRODUCTION

The production of specific secretory Ig A by the adenoid and middle ear mucosa constitute an important part of the local immunological factor protecting the middle ear against invasion of both viruses and bacterial pathogens[1]. Ig A provides the dominant surface response to polysaccharide and lipopolysaccharide antigens but once the mucosa has been breached, most protection is provided by Ig G_2. It has an important role as a neutralizing antibody in the prevention of extensive tissue damage by this destructive toxin of a common respiratory pathogen[3,4,5]. The aim of this study was to determine the serum level of Ig A in the serum and the middle ear secretion in suppurative otitis media and evaluate the difference by comparing between acute (ASOM) and chronic (CSOM).

MATERIALS AND METHOD

SUBJECTS

Following Approval of study proposal by the Institution Review Board of the University of Ibadan/University College Hospital Ibadan and obtaining informed consent from the parents, recruitment was commenced. The inclusion criteria were children under the age of 12 years with acute and chronic otitis media using the reference of 3 months as cut off for acute OM[6]. All the patients were recruited from the General Out-patient department and the Otorhinolaryngology outpatient Clinic of the University College Hospital, Ibadan. The patients had history taken, and examination of the ear, nose and throat was done with a hand held otoscope and shirom lamp and head mirror. The controls were selected from children without otitis media.

SAMPLE COLLECTION

Middle ear effusion was collected by aspiration method using a Pasteur pipette into microfuge bottle and 5 millilitres of venous blood was collected from the antecubital vein into a non-heparinized bottle and spun at 1500xg for ten minutes. After clot extraction, the serum was separated and stored at -80°C.

QUANTIFICATION OF IMMUNOGLOBULIN IG A

Ig A was quantified by the single radial immunodiffusion method. A 3% noble agar was prepared in phosphate buffered saline (PBS, pH 7.2) containing 0.2% sodium azide. One milliliter of each antisera (anti-human immunoglobulin class) was mixed with 7ml of PBS. Eight milliliters of the 3% noble agar was thoroughly mixed with the diluted antiserum. The mixture was carefully poured on a glass plate placed on a leveler avoiding the formation of air bubble. The agar-antiserum mixture was allowed to set and wells of 3 mm in diameter were made in the agar with a circular metal punch. The punched agar was carefully removed from the plate with the smooth edge of pipette attached to a vacuum pump. Several dilutions(25%, 50%, 100% and 200%) of the standard serum were prepared in PBS. Using a 5ml micro-dispenser the sera middle ear effusion and standards were applied to the punched wells. The plates for Ig A estimation were incubated for 18 hours. The diameter of the precipitation ring was measured along two perpendicular diagonals to the nearest 0.1mm using eye precision viewer. The standard curves for the various classes of immunoglobulins were plotted on a semi-log graph paper and the concentrations of the test and control samples were read off the standard curve.

STATISTICS

The main outcome variables were the Ig A levels in serum and middle ear secretion. The data was initially explored using the Stata software, variables were analysed by unpaired t-test both for equal and unequal variance using the variance ratio function of the Stata software to determine the appropriate use of the Satterthwaite's correction for the degrees of freedom. Level of statistical significance was at $p< 0.05$ for all the analyses.

RESULTS

The subjects and control were made of 30 males and 22 females, between the ages of 6 months and 9 years, mean of 6 years(SD =3.26).

Paired sera and middle ear secretion (MES) from 37 subjects with suppurative otitis media comprising of 20 CSOM and 17 ASOM and sera of 15 control were analysed. The mean serum level of Ig A were control 36mg/dL, ASOM 46.1mg/dL, CSOM 41mg/dL, while the MES level of Ig A were ASOM 228.3mg/dL and CSOM 85.4mg/dL. table 1.

The mean MES : serum ratio was 4.95 for ASOM and 2.1 for CSOM.

The ratio of serum level of control to ASOM was 1.3 while control to CSOM is 1.14.

Multivariate analysis using unpaired t-test to compare the mean revealed significant difference between the MES level of ASOM and CSOM (P= 0.0056) but no significant difference between serum level of ASOM and CSOM(P=0.57), serum level in control and otitis media subjects (ASOM, P=0.25, CSOM, P=0.60).

Figure 1

Table 1: Values Ig A level of serum and middle ear secretion

Ig A level (mg/dL)	Control Serum(n=15) (mg/dL)	ASOM Serum(n=17) (mg/dL)	ASOM MEE(n=17) (mg/dL)	CSOM Serum(n=20) (mg/dL)	CSOM MEE(n=20) (mg/dL)
Range	0 - 61.50	0 – 45.50	130 – 326.3	0 – 55.1	55.0- 115
Mean	36	46	228.3	41	85.4
Median	31.5	42.6	192.0	40	81
Standard Deviation	2.6	1.5	1.6	2.6	4.8

DISCUSSION

In this study the finding of reduced serum and middle ear concentration of Ig A in CSOM compared to ASOM and the significant difference between the MES levels in ASOM and CSOM (P= 0.0056) suggests that reduced secretion may predispose to chronicity. Jónsson et al[5] have reported that sustained low levels of IgA proved the strongest single indicator of susceptibility for recurrent otitis media (P=0.008) and respiratory tract infections (P=0.02), confirming our finding of low MES Ig A in CSOM compared to ASOM.

However, the MES : serum Ig A ratio of 2.1 - 4.95 in ASOM and CSOM showed higher concentration of Ig A in the middle ear than serum. This finding of an exaggerated middle ear concentration in both acute and chronic SOM in this study suggest that Ig A production possibly originates from middle ear mucosa secretory response to inflammatory stimuli. Sloyer et al[6,7] also reported that the occurrence of IgA antibody in MES and its absence or gross reduction in simultaneously drawn serum was as an indicator of local antibody production. Of the 401 assays performed in their

study, 41 instances of IgA antibody exclusively in MES were found.

Bernstein[1] and others[7,8,9,10, 11] reported that Ig A is produced in the adenoid and nasopharynx and directed against both viruses and bacterial pathogens in a genetically controlled fashion1. Pichichero et al[9] and Paton et al[12] also found IgA class antibodies to the capsular polysaccharides of S. pneumoniae were detected more often in the middle ear and occurred independently of IgA antibody in serum. They also found correlation with the presence of the secretory component in pneumococcal antibody, indicating local production of IgA antibodies. Children with pneumococci found in MES samples developed nasopharyngeal IgA antibody responses to capsular polysaccharides more often than did children with pneumococci found only in the nasopharynx or not at all, indicating that the presence of S. pneumoniae in the middle ear was stimulative for nasopharyngeal antibody production. Similarly, Virolainen et al[4,8]studied nasopharyngeal aspirate samples of 120 children with acute otitis media and detected IgA class antibody in 93%.

In this study, we used middle ear secretion samples for the estimation of the immunoglobulin similar to the work of Virolaein et al[4, 8, 11]. Our finding of Ig A in the middle ear secretion may suggest responses to nasopharyngeal possible middle ear inflammation associated with ASOM and CSOM.

We conclude that middle ear Ig A secretion is higher in ASOM than CSOM, thus reduced secretion may be associated with chronicity. The monitoring Ig A assay may be useful index of determining likely progression to chronicity, however, further study is still needed to establish this hypothesis.

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