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Retrospective Invitro Study of Effect of Ciprofloxacin and Azithromycin againt Pseudomonas Isolated in Chronic Suppurative Otitis Media

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Abstract :

Background : Chronic ear discharge is one of the common presentations to otorhinolaryngologist. With most common organism isolated being pseudomonas, which is resistant to commonly used antibiotic, particularly those used topically/orally. Azithromycin is not been used as an anti pseudomonal agent.

Aims : In this study we compare in-vitro effect of commonly used antipseudomonal agent, ciprofloxacin, with that of azithromycin.

Materials and Methods : Retrospective review of laboratory records of ear swabs submitted for culture, in SS Institute of Medical Science and Research Centre, with culture report showing pseudomonas organism with sensitivity done, for both ciprofloxacin and azithromycin, were considered.

Statistical Analysis : Comparative study with fisher's exact test.

Results : 48 ear swabs were received by the laboratory for processing showed, susceptibility of Pseudomonas to ciprofloxacin was 47.9% and that of azithromycin was 52.1%. There was no significant variation between antipseudomonal effect of ciprofloxacin and azithromycin with p value =0.065.

Conclusions : Azithromycin has antipseudomonal property par equivalent to ciprofloxacin, with further study mandated.

Key words: Otitis media, Pseudomonas, Ciprofloxacin, Azithromycin, antimicrobial agents, Microbial Sensitivity Tests.

Introduction: Chronic suppurative otitis media (CSOM) is, defined as a chronic inflammation of the middle ear and mastoid cavity, which presents with recurrent ear discharges or otorrhoea through a tympanic perforation. It is an important cause of preventable hearing loss, particularly in the developing world. Indian prevalence of CSOM is7.8%.[1]

CSOM is caused by various organism, Pseudomonas being the commonest.[1,2] Ciprofloxacin is one of the common anti pseudomonal antibiotic which can be used topically, orally and also parental route. Its antipsuedomonal action is well establish and studied. [1-3] Azithromycin also have antipseudomonal property[4,5] but it is not well studied. So this article emphasizes on comparing the two drugs that is ciprofloxacin and azithromycin in having antipseudomonal property, in CSOM patients.

Methodology :

Retrospective review of laboratory records of ear swabs submitted for culture, in SS Institute of Medical Science and Research Centre (SSIMS&RC), Davangere, from June 2012 to December 2014, were considered.

Inclusion Criteria :

1. Patients with more than three months of ear discharge.[1]
2. Pure Pseudomonas culture in ear swab.
3. Both ciprofloxacin and azithromycin sensitivity study conducted in same sample.

Exclusion Criteria :

1. Ear discharge less than 3 months.
2. Culture with only ciprofloxacin sensitivity done.
3. Culture with only azithromycin sensitivity done.

Study Design : Retrospective study.

Specimen collection and processing : Patients in the age group 5 to 75 years, who met the inclusion criteria, formed the study subject. Ear swabs were taken using dry sterile swabs, by the Department of Otorhino-laryngology and transported immediately to department of Microbiology in SS Institute of Medical Science and Research Centre, Davangere, India. The swabs were plated on MacConkey agar, Blood agar and Chocolate agar and incubated aerobically at 37°C for 24 hours. The

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organisms were identified according to standard microbiological procedures.[6] All isolated strains were tested for susceptibility to antibiotics, including ciprofloxacin and azithromycin, on Mueller Hinton Agar using Kirby Bauer disc diffusion method. Results were interpreted using Clinical Laboratory Standards Institute (CLSI) guidelines.[7] All dehydrated media, reagents and antibiotic discs were procured from Microxpress, Tulip Diagnostics (P) Ltd., Goa, India.

Results :

Out of 170 ear swabs, with pseudomonas growth, collected during study period, 48 met the inclusion criteria and were considered. Among the study population 30(62.5%) were male and 18(37.5%) were female (table 1). Demographic details of the study population showed that, the mean age group of patients affected was found to be 28.62 years (ranging from 5 to 75 years), age group most commonly affected were less than 30 years which accounts to 31 patients(64.6%) and above 30 years accounted 17 patients(34.4%) (Table 2). Sensitivity and resistance pattern among the study participants showed that 23 patients (47.9%) were ciprofloxacin sensitive and 25 patients (52.1%) were ciprofloxacin resistant (table 3) similarly, 33 patients (68.8%) were azithromycin sensitive and 15 patients (31.2%) were azithromycin resistant (table 4). Fisher's exact test applied for the comparing the effectiveness between two drugs showed the p value = 0.065, which is not significant. It was also found combined sensitivity of azithromycin and ciprofloxacin was found in 19 patients (39.58%) and combined resistance of azithromycin and ciprofloxacin was found in 11 patients (22.92%). So combined sensitivity or resistance pattern noted was 30 of the 48 samples (62.5%) (table 5). Comparison of antibiotic sensitivity of ciprofloxacin in age group less than 30 years and those more than 30 years it did not show any significant difference in sensitivity pattern with p value= 1.0(table 6), similar result was also found in azithromycin with p value= 0.749(table 7).Drug sensitivity and resistant pattern of ciprofloxacin and azithromycin is depicted in graph 1.

Discussion :

Chronic suppurative otitis media is persistent inflammation of the middle ear or mastoid cavity. Synonyms include "chronic otitis media", chronic mastoiditis, and chronic tympanomastoiditis. CSOM is characterized by recurrent or persistent ear discharge (otorrhoea through a perforation of the tympanic membrane, lasting more than 3 months. CSOM usually begins as a complication of persistent acute otitis media (AOM) with perforation in childhood. Typical findings may also include thickened granular middle-ear mucosa and mucosal polyps. Occasionally, CSOM will be associated with a cholesteatoma within the middle ear. CSOM is differentiated from chronic otitis media with effusion, in which there is an intact tympanic membrane with fluid in the middle ear but no active infection. CSOM does not include chronic perforations of the eardrum that are dry, or only occasionally discharge, and have no signs of active infection. Cholesteatoma is an abnormal accumulation of squamous epithelium usually found in the middle ear cavity and mastoid process of the temporal bone. Granulation tissue and ear discharge are often associated with secondary infection of the desquamating epithelium. Cholesteatoma is most often detected by careful otoscopic examination in children or adults with persistent discharge that does not respond to treatment. The incidence of chronic suppurative otitis media (CSOM) appears to depend on race and socio-economic factors. Socio-economic factors such as poor living conditions, overcrowding, poor hygiene and nutrition have been suggested as a basis for the wide spread prevalence of CSOM.[1,3]

Pseudomonas species was the most commonly isolated organism in CSOM.[8,9] Pseudomonas aeruginosa (PA), is an aerobic, nonfermenting Gram-negative rod bacterium, is one of the most troublesome bacteria in hospital-acquired infections. Infections caused by PA can cause various diseases, including otitis externa, osteomyelitis, meningitis, endocarditis, pneumonia, urinary tract infection and septicemia. It can colonize various sites of the human body, including the sputum, cornea, nasal mucosa and wet skin, causing a range of diseases from minor skin infections to fulminant septicemia.[10] It is a common environmental organism usually found in warm and moist environment, and is known to colonize the external auditory canal. It is commonly associated with otitis externa and chronic superative otitis media.[7]

Age-wise distribution of prevalence of culture positive cases of CSOM revealed that it was more common in young populations. Most developing countries have predominantly young populations in whom CSOM is most prevalent. CSOM can affect paediatric and adult groups.[8,9] In our study, CSOM was found mostly among young adults, with a mean age of 28.6 years.

Gender distribution of the disease is widely variable in different studies. Females were more commonly affected than males on study done by Tahira Mansoor et al, and it is also supported by a study carried out in Singapore. In contrast to this some studies showed opposite trend reported male 57.3% and female 42.7%and this can be

due to geographical variation.[14] In this study, disease was more common in males (62.5%) than in females (37.5%) and the male: female ratio was found to be 1.67:1. This was further supported by six months study done by Raghu Kumar KG et al. at the same locality, which showed male to female ratio of 1.65:1.[12]

Ciprofloxacin is a quinoline compound which is highly active against a broad spectrum of pathogenic bacteria, particularly against gram-negative bacteria. It acts on the A subunit of DNA gyrase, which inhibits and affects DNA supercoiling, resulting in inhibition of DNA replication and possibly transcription[.3,15,16]

Pseudomonas sensitivity to ciprofloxacin varies widely in with location, sensitivity to Pseudomonas the values include ,93% in Ghana[11], 85% in Karachi[14], 83.7% in Turkey.[17] In this study ciprofloxacin sensitivity to pseudomonas was 47.9% comparable to that of Raghu Kumar et al. which was 47.37 %.[12] There is a gross difference in sensitivity, compared to the other studies. This value shows the growing trend of pseudomonas resistance in this locality. The major benefit of the ciprofloxacin treatment is: it is cost effective, minimal side effects, this is available as topical preparation, oral preparation and also as injectable agent.

Azithromycin is used as oral preparation and injectable is also available. Azithromycin can also be used topically.[18,19] There were various mechanism proposed for the antipseudomonal property of azithromycin by various authors. Experiments by Tatida K et al. confirm that exposure time is a critical factor for macrolide-mediated bactericidal activity against Pseudomonas. Specifically results indicate that an exposure period of 48 h or longer allows the macrolide antibiotics erythromycin, clarithromycin and azithromycin, to demonstrate bactericidal activity against *P.* aeruginosa at concentrations far below the minimum inhibitory concentration(MIC) This study also indicated that Azithromycin inhibits protein synthesis before any decrease in viability can be observed. Azithromycin was observed to accumulate intracellularly in a time-dependent manner. These results suggest that macrolide exposure induces changes in the cell surface structures of bacteria, which may in turn facilitate macrolide entry and allow antibiotic to accumulate within the cell.[4] Imperi et al. has also explained in detail the pleiotropic effects of sub-MIC Azithromycin on *P.*aeruginosa are mainly due to interaction with the ribosome and interference with protein synthesis.[14,15] Mizukane et al. study Vitro Exoenzyme-Suppressing Activities of Azithromycin and Other Macrolide Antibiotics which included erythromycin , roxithromycin, and rokitamycin showed that Azithromycin has the strongest virulence-suppressing effect. This study showed that sub-MICs of Azithromycin inhibit exotoxin A, total protease, elastase, and phospholipase C production by P. aeruginosa without affecting either the growth of P. aeruginosa or its total protein production.(5) Kawamura-Sato et al. study on Erythromycin, clarithromycin, and azithromycin at subinhibitory concentrations (sub-MICs) suppressed the expression of flagellin dose dependently. Azithromycin had the strongest inhibitory effect on the expression of *P.aeruginosa* flagellin, Studies indicated that sub-MICs of Erythromycin, clari-thromycin, and azithromycin may be clinically useful for the treatment of patients with *P. aeruginosa* infections through inhibition of the biofilm formation.[22-24]

Bacterial biofilms play a relevant role in persistent infections, which are rarely eradicated with antimicrobial therapy Lutz et al. study on total of 64 isolates showed biofilm inhibitory concentration (BIC) results were consistently higher than those obtained by the conventional method, minimal inhibitory concentration, (MIC) for most antipseudomonal agents tested (ceftazidime: P = 0.001, tobramycin: P = 0.001, imipenem: P < 0.001, meropenem: P = 0.005). When macrolides were associated with the anti-pseudomonal agents, the BIC values were reduced significantly for ceftazidime (P<0.001) and tobramycin (P<0.001), regardless the concentration of macrolides. Strong inhibitory quotient was observed when azithromycin at 8 mg/L was associated with all anti-pseudomonal agents tested in biofilm conditions.[25]

Number of clinical studies have demonstrated that patients suffering from both intermittent and chronic P. aeruginosa infection, e.g., cystic fibrosis, chronic obstructive pulmonary disease and diffuse panbron chiolitis, benefit from azithromycin treatment[20] Beneficial effects have so far been documented in cystic fibrosis patients treated with azithromycin for many months, while reduced efficacy was associated with longer treatment duration .[21,26]

❖ No article was found comparing antipseudomonal activity between azithromycin and ciprofloxacin, in CSOM. It was surprising to find lot of CSOM due to pseudomonas showing culture sensitivity to azithromycin, in our outpatient department. So this study was planned. Detailed study of antipseudomonal property was evaluated for both ciprofloxacin and azithromycin, theoretically and also through retrospective study of data. Our study showed that there was no significant difference between usage of ciprofloxacin and azithromycin (p value = 0.065), which

means azithromycin have antipseudomonal property par equivalent to ciprofloxacin. There is no significant variation sensitivity of drug, with usage based on age, age group less than 30 years and more than 30. It was also found that combined sensitivity or resistance of ciprofloxacin and azithromycin was noted 62.5% of the patients.Since this is a retrospective study and considering the limitations of this study following studies are recommended, which includes:

- ❖ Large prospective invitro
- ❖ Invivo randomized control trial.
- ❖ To study the association between ciprofloxacin resistance and increased sensitivity to azithromycin, in various tissue samples.
- ❖ Topical effect of azithromycin compared to ciprofloxacin with or without oral drug combination.
- ❖ To know if there is a synergistic effect on combined use of ciprofloxacin and azithromycin.
- ❖ Pharmacological interaction between ciprofloxacin and azithromycin when used in various routes.
- ❖ Action of ciprofloxacin, azithromycin and combination of drugs against biofilm due to pseudomonas.
- ❖ Like in cystic fibrosis, to find if there is any role of azithromycin in controlling pseudomonas infection.
- ❖ To compare azithromycin sensitivity to pseudomonas in various environment, age, sex and also different body sample like in ear discharge, tracheostomy stoma discharge, nasal discharge, sputum, urine, blood etc.

Conclusions :

Males are predominantly involved by CSOM due to pseudomonas infection and young adults are more susceptible. Azithromycin has antipseudomonal property par equivalent to ciprofloxacin, invitro, with further study mandated. There is no difference in sensitivity pattern of azithromycin or ciprofloxacin when in used in different age group. There is increased in trend of ciprofloxacin resistance to pseudomonas, on ear discharges, in Davangere.

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