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Research Article

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A Case Control Study Performed in Karachi on Inflammatory Markers by Ciprofloxacin and Co-Amoxicillin in Patients with Chronic Suppurative Otitis Media

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ABSTRACT

Introduction: Chronic suppurative otitis media (CSOM) is middle ear infection, perforation and inflammation usually with purulent middle ear discharge. Different immune markers such as IgE and interferon were proved to be elevated in CSOM.

Aim/Objective: The aim of the study is to evaluate of these inflammatory biomarkers (IL6, IL8 and TNF gamma) in patient of CSOM to suggest future therapy of chronic infections with inflammations.

Methodology: This is a case-control study conducted in ENT ward in a tertiary care hospital in Jinnah Post Medical centre, Karachi during November 2017 to May 2018. Patients or healthy volunteers were divided into two groups as G1 (without any disease) and G2 (CSOM patients without treatment), G3 (CSOM patients treated with co-amoxicillin) and G4 (CSOM patients treated with ciprofloxacin). The serum was subjected for the quantitative measurement of IL6, IL8 and TNF Gamma by ELISA Kit method via antibody-antigen-enzyme complex formation and measured at 450 nm.

Result: Study shows that IL6, IL8 and TNF gamma concentration was significantly high in G2 (positive control), G3 (ciprofloxacin) and G4 (co-amoxicillin) in contrast with G1 (negative control). Moreover, IL6 concentration was significantly low in G3 (ciprofloxacin) and G4 (co-amoxicillin) as compare to G2 (positive control).

Conclusion: Elevated levels of IL6, IL8 and TNF gamma in CSOM showed alteration of immune response in chronic suppurative otitis media. Moreover, anti-inflammatory role of ciprofloxacin and co-amoxicillin in CSOM may provide additional benefit beside attenuation of infection.

Key Words: Chronic suppurative otitis media, Ciprofloxacin, Co-amoxicillin, Immune markers, ELISA Kit method, Anti-inflammatory role

INTRODUCTION

Chronic suppurative otitis media (CSOM) refers the chronic infection alongwith inflammation of middle ear. CSOM is commonly associated with recurrent purulent middle ear discharge. Although it is not typically linked with pain but due to severe inflammation patients have to compromise quality of life and may suffer even fever, dizziness or vertigo etc. Otorrhea is one of the leading complications of CSOM. Study showed that 73 % of CSOM patients had intermittent drainage but 27 % had continuous otorrhea for approximately one year. Another problem associated with CSOM is hearing loss. CSOM is one of the leading cause of hear loss all over the world and may lead to threshold of hearing less than in 50-60 percent of patients with CSOM. [1]

Immune system play role in to play a role in CSOM as it does in many chronic infections. Studies showed that environmental allergens may play an important role in CSOM. Immunoglobulin E (IgE) in serum of test group was higher than control group. In a study 80.00% of test group participants were allergy positive while 47.00% patients with AOM showed positive test and only 33.00% healthy individuals were allergy positive.[2] Hence, literature provides enough evidences to support cytokines roles in CSOM. Altered levels of cytokines such as interleukin 6 (IL6), interleukin 8 (IL8) and

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tumor necrosis factor alpha (TNF-α) can be used as biomarkers of different diseases.[3]

Interleukin-6, encoded as IL-6, plays important role as a pro and anti-inflammatory secretion of T cells and macrophages in infections and inflammation. It acts as both myokine and cytokine in human body but reported to show resistant against some bacterium in animal model. It is responsible to stimulate pro-inflammatory cytokine when produced by osteoblast and tunica media.[4] Similarly, IL8 is also an important part of inflammatory system. It is also called neutrophil chemotactic factor due to its chemotaxis property in neutrophils but it has other targets too. It contributes in phagocytosis and exocytosis through different processes such as histamine release or increasing intracellular Ca^{2+}. IL-8 is involved in pathophysiology of chronic infection with allergy. It is an important part of CXC chemokine system besides other ten members, very close to chromosomes.[5] TNF gamma has its role in different infection in animal model and it can be a future target of chronic infections with inflammation.[6]

The aim of the study is to evaluate of these inflammatory biomarkers (IL6, IL8 and TNF gamma) in patients of CSOM and effects of ciprofloxacin and co-amoxicillin on it.

METHODOLOGY

This is a case-control study conducted in ENT ward in a tertiary care hospital in Karachi during November 2017 to May 2018. Patients or healthy volunteers were divided into two groups as G1 (without any disease) and G2(CSOM patients without treatment), G3(CSOM patients treated with co-amoxicillin) and G4 (CSOM patients treated with ciprofloxacin).

Ethical approval: The ethical approval (JSMU/IRB/2017-66) was declared by concerned Institutional Review Board for clinical research in accordance with the relevant regulations.

Inclusion criteria: Patients of both the genders, adults (16-65±1 years), having unilateral or bilateral ear perforation, diagnosed with CSOM with otoscopy, with and without cholesteatoma.

Exclusion criteria: Paediatric population and patients above 65±1 years with the history of neurological or psychiatric disorder, cardiac arrest or auto-immune disorder.

Sample size calculation: the minimum sample size was calculated (n=75) with the help of OPEN-EPI, keeping 5.2% prevalence and 95% confidence interval, taking 5% margin of error but we enrolled 160 patients in the current study.[7]

Sample Collection: Blood (3ml) was collected from patients and volunteers which was collected subjected to the centrifugation to obtain serum. Serum was stored at -70°C in eppendorff until quantified with the help of ELIZA when sample size fulfilled.

Determination of IL6, IL8 and TNF Gamma by Elisa Kit: The serum was subjected for the quantitative measurement of IL6, IL8 and TNF Gamma by ELISA Kit method via antibody-antigen-enzyme complex formation and measured at 450 nm.

RESULT

The levels of IL-6 presented as mean with +S. D proved significantly different (p= 0.00, F value 11.76) when one way ANOVA was applied. Group comparison was done by Post Hoc (Tukey) test (table 1, figure 1) which shows that IL6 concentration was significantly high in G2 (positive control), G3 (ciprofloxacin) and G4 (co-amoxicillin) in contrast with G1 (negative control). Moreover, IL6 concentration was significantly low in G3 (ciprofloxacin) and G4 (co-amoxicillin) as compare to G2 (positive control).

Similarly, effects of CSOM on IL 8 serum levels found to be significantly different with *p* value= 0.00 and F value =7.76. IL 8 serum levels presented as mean +SD (table 1 and figure 2). effects of antibiotics G3 (ciprofloxacin) and G4 (co-amoxicillin) showed significantly low levels of IL8 as compare to G2 (positive control) but higher than G1 (negative control).

The study presented (table 1, figure 3) significant difference (p=0.00) with F-value of 14.48 when one way ANOVA was applied to evaluate the effects of CSOM on TNF gamma beta levels. However, Post Hoc (Tukey) test showed significantly higher levels in G2 (positive control), G3 (ciprofloxacin) and G4 (co-amoxicillin) in contrast with G1 (negative control) but also significant difference between G2 (positive control) and groups treated with antibiotics G3 (ciprofloxacin) and G4 (co-amoxicillin).

DISCUSSION

High serum levels of some cytokines such as interleukin 6, interleukin 8 andlymphotoxin beta can be associated with poor immune function in chronic infection.[3,4,5,6] Therefore, in present study these cytokines were measured as biomarkers of poor porosis like other chronic infections. In addition to this, these markers also found to be altered or elevated in some neurological or depressive disorders. Hence, it may also reflect the possible association with depression induces by CSOM as reported in earlier study.[2,8]

Significant increase in IL6 levels showed that IL6 might contribute in prognosis of CSOM infection. Studies showed

inflammatory role of IL6 in different infection by secretion macrophages or its role on the release of other cytokines.[3-6] Its high levels were also reported to be associated with depression and in cognitive control by brain derived neurotrophic.[8] Therefore, its increased levels in CSOM patients might have its role in CSOM or in provoking depression in CSOM patients.

Similarly, another interleukin 8 was found in significantly higher levels. This may be due to its role in the progression of CSOM infection. Interleukin 8 was reported to effect chemotaxis, angiogenesis, and phagocytosis and histamine release and in different neurological disorders.[3] Hence, it may play effective role in contributing allergy and depression in CSOM patients. In the same way LTB indicated poor prognosis in several chronic infection,[8] so as in CSOM in current study.

Therefore, IL6, IL8 and TNF gamma might be responsible of worsening of inflammation, infection or depression in CSOM.

The treated groups of ciprofloxacin (G3) and co-amoxicillin (G4) showed significant decreased of IgE, LTB, IL6 and IL8 from untreated patients (G2). Similarly, the significant difference from G1 (negative control) from treated of ciprofloxacin (G3) and co-amoxicillin (G4) showed that ciprofloxacin and co-amoxicillin may help to normalize LTB levels but not up to the normal status. In addition to this, ciprofloxacin (G3) proved to produce better results than co-amoxicillin. Study showed anti-inflammatory effects of ciprofloxacin on IL 6, tumor necrosis factor alpha and other pro and inflammatory cytokinein serum of patients with sepsis. [9]Roles of amoxicillin on decreasing inflammatory markers (IL6 and INF gamma) in community acquired disease is also established. [10]Therefore, ciprofloxacin and co-amoxicillin may provide beneficial effects to decrease elevated inflammatory markers.

CONCLUSION

Elevated levels of IL6, IL8 and TNF gamma in CSOM showed alteration of immune response in chronic suppurative otitis media. Moreover, anti-inflammatory role of ciprofloxacin and co-amoxicillin in CSOM may provide additional benefit beside attenuation of infection.

In future work, development of fully functional decentralized application (blockchain technology) will be done to develop and maintain metadata of medical history of patients with CSOM. It will be helpful in predicting critical condition of patients with compromised immunity and CSOM when algorithms will be applied.

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Conflict of Interest: None to declare.

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Authors' Contribution: All authors contributed equally.

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Table 1: IL 6, IL8 and TNF gamma levels in participants of G1 (negative control) from G2 (positive control), G3 (ciprofloxacin) and G4 (co-amoxicillin) groups

Groups	IL 8 (pg/ml) Mean±SD	p value	IL 6 (pg/ml) Mean +S. D	p value	LT Beta (pg/ml) Mean ±S. D	p value
G1: (negative control)	24.03±9.28	_	0.741±0.48	_	9.37±1.8	_
G2: (positive control)	199.68±12.81	p< 0.05	3.21±0.61	p< 0.05	90.62±12.5	p< 0.05
G3: (ciprofloxacin)	40.64±11.82	p< 0.05	2.39±01	p< 0.05	29.95±12.1	p< 0.05
G4: (co-amoxicillin)	184.4±43.1	p< 0.05	2.5 ±0.82	p< 0.05	39.34±11.1	p< 0.05

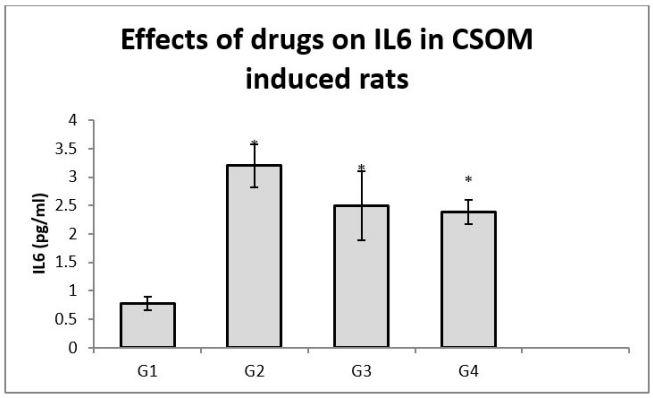

Figure 1: IL 6 presented as mean concentration in serum of participants of G1 (negative control) from G2 (positive control), G3 ciprofloxacin (1000 mg) and G4 co-amoxicillin (1000mg) (p<0.05).

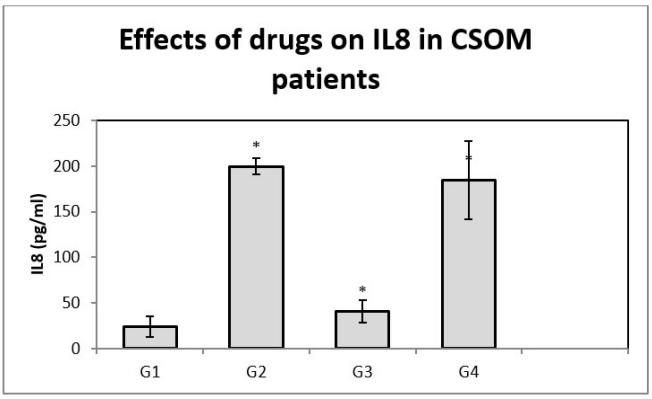

Figure 2: IL 8 presented as mean concentration in serum of participants of G1 (negative control) from G2 (positive control), G3 ciprofloxacin (1000 mg) and G4 co-amoxicillin (1000mg) (p<0.05).

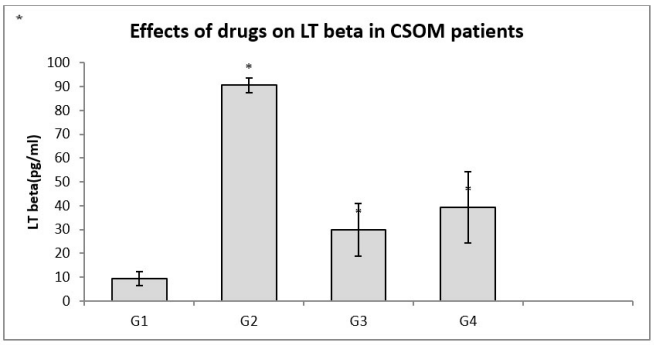

Figure 3: TNF gamma presented as mean concentration in serum of participants of G1 (negative control) from G2 (positive control), G3 ciprofloxacin (1000 mg) and G4 co-amoxicillin (1000mg) (p<0.05).