Biochemical effects of ear infections by Pseudomonas aeruginosa on rabbits

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A B S T R A C T

Ear infections (particularly, otitis media) is a prevailing and common infection in developing countries causing local damage and threatening complications. Pseudomonas aeruginosa is the most common pathogen causing chronic suppurative otitis media (CSOM) and malignant otitis externa. The objective of this study is to identify incidence of Pseudomonas aeruginosa involved in ear infections and associated biochemical parameters which may be changed. External auditory canal in rabbit ears was inoculated with (10^6) colony-forming units (CFU) of Pseudomonas aeruginosa or left as sterile controls for eight weeks. There are significantly decrease results at ($P<0.05$) of Interleukin-2 and significant increase results of Interleukin-9 and Tumor Necrosis Factor-alpha (TNF-α) in acute or chronic infections. Moreover, histopathological changes of ear, kidney and liver tissues were also observed. Evaluation of these parameters were needed to use of specific blocking agents to inflammatory mediators with the aim of discovering new treatment options for chronic otitis media, and to help the physicians in diagnosis these diseases in elderly stages and try to reduce the pain and suffering associated with otitis media.

Keywords: Otitis Media (OM), Pseudomonas Aeruginosa, Interleukins.

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1. INTRODUCTION

Otitis is a general term for inflammation or an infection in human ear. It is divided as otitis externa, which involves outer ear and ear canals. Otitis media involves middle ear. Otitis interna involves inner ear (Rashid et al., 2014). Otitis media (OM) is one of the primary conditions for which antibiotics are prescribed in the United States (McCaig et al., 2002). It can be acute or chronic. The acute form usually associated with the infection in the upper respiratory tract whereas persistent form is known as chronic suppurative otitis media (CSOM). The chronic form is still a major problem in developing countries like Pakistan. It is more common in children belonging to lower socioeconomic group. Most common microorganisms found in CSOM are Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumoniae, Escherichia coli, Aspergillus spp and Candida spp but these organisms vary in various geographical areas (Salam et al., 1997). Otitis media with effusion (OME), inflammation of the middle ear, is the commonest cause of hearing impairment in children and the commonest reason for surgery in children. Both chronic and recurrent forms of OM are known to have a significant genetic component (Daly et al., 2004). and formation of mucus glands occurs (Goycoolea., 2001, Tos and Thomasen., 2002). The immune system responds to injury or irritation through an innate cascade known as inflammation. There are many cellular and biochemical events occur as a result of tissue injury that responsible for the outcome of inflammation and release inflammatory

mediators, which include proteins, peptides, glycoproteins, cytokines, arachidonic acid metabolites (prostaglandins and leukotrienes), nitric oxide, and oxygen free radicals. These compounds are produced by epithelial cells, endothelial cells, and infiltrating inflammatory cells. Inflammatory mediators are a double-edged sword, having the potential to fight off infection, but also to damage the host (Juhn et al., 1994). Inflammatory mediators important in Otitis Media (OM) are produced by infiltrating immune cells such as neutrophils, monocytes, and lymphocytes. In addition, local cells such as keratinocytes and mast cells have been shown to produce inflammatory mediators. It is also coming to light that besides the middle ear, the inner ear tissues are able to produce inflammatory cytokines and use NFκB activation (Ghaheri et al., 2007). Otitis Media is frequently caused by bacteria which enter the middle ear from the nasopharynx via the eustachian tube. In cases of bacterial otitis media, the source of bacteria is currently believed to be the nasopharyngeal tonsils (adenoids). However, cases of sterile OM are not uncommon and the mechanism of this condition is currently unclear. Endotoxin, a component of bacterial cell walls, is believed to be responsible for initiating inflammation in the middle ear. Endotoxin is a potent inducer of various inflammatory mediators, as well as a modulator of the immune response, and stimulates local macrophages to produce tumor necrosis factorα (TNFα) and interleukin-1β (IL-1β). In addition; keratinocytes have the ability to produce many soluble mediators independently from immune cells in response to injury, including TNFα, ILβ, IL-1, IL-6, and IL-8 (Hansen et al., 1991).

2. MATERIAL AND METHODS

2.1. Rabbits

Twelve male rabbits weighting about (1kg-1.5kg) and average body weight (2-4months) were used in the experimental investigations of this study. Rabbits were obtained from "The Laboratory Animals Research Center", Faculty of Veterinary Medicine, Benha University, and housed in separate wire mesh cages, exposed to good ventilation, humidity and to a 12-hr light/dark cycle. Constant supplies of standard pellet diet, fresh and clean drinking water were supplied ad-libitum. The animals were left for 15 days for acclimatization prior to the beginning of the experiment, and kept at constant environmental and nutritional conditions throughout the period of the experiment.

2.2. Bacteria

Strain of Pseudomonas Aeruginosa obtained from the laboratory of department of bacteriology, Faculty of veterinary Medicine, Benha University, Egypt. Bacteria were extracted from a reservoir following the instructions of the manufacturer and cultured on blood agar for 24 h at 37° C. After incubation, the bacteria were suspended in sterile saline at a concentration of 10^6 colony-forming units (CFU)/ml. The concentration of bacteria was confirmed by means of a standard colony plate count (Seth et al., 2013).

2.3. Experimental Design

Group (1): Normal Control Group: Comprised 4 rabbits, no bacteria inoculated, provided only with a constant supply of standard pellet diet and plenty of fresh, clean drinking water ad-libitum.

Group (2): Infected Group: Comprised 8 rabbits, which inoculated in one ear canal by pseudomonas aeruginosa (10^6) colony-forming units (CFU)/ml, and then leaves this group about two months without treatment.

2.4. Sampling

Blood samples were collected after 2 & 4 & 8 weeks from all animals groups (control and experimental groups). Samples were collected from ear vein of all animal groups and were centrifuged at 3000 rpm for 30 minutes to separate serum. Serum was kept in deep freezer till use for analysis of

biochemical parameters: IL-2, IL-9 and TNF-α.

2.5. Statistical analysis

The obtained data were analyzed and graphically represented using the statistical package for social science (SPSS, 13.0 software, 2009), for obtaining mean and standard deviation and error. The data were analyzed using one-way (ANOVA) to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparison among the groups for testing the inter-grouping homogeneity.

Data in Table (1) represented that there is a significant difference in serum IL-2 in group (G2) compared with group (G1) control either at first and second period of infection by pseudomonas aeruginosa. Data in Table (2) represented that there is a significant difference in serum IL-9 in group (G2) compared with group (G1) control either at first and second period of infection by pseudomonas aeruginosa. Data in Table (3) represented that there is a significant difference in serum TNF-α in group (G2) compared with group (G1) control either at first and second period of infection by pseudomonas aeruginosa.

3. RESULTS

Table (1) Effects of Pseudomonas aeruginosa infection (10^6 colony-forming units (CFUs)/ml) on serum interleukin-2 (IL-2) concentrations in male rabbits

Parameter	control	Weeks post-infection by pseudomonas		
		2 weeks	4 weeks	8weeks
IL-2 (pg/ml)	89.17 ± 6.15^{b}	41.28 ± 5.55^{a}	49.88 ± 14.25^{a}	70.10 ± 12.01^{ab}

Data are presented as (Mean ± S.E), S.E = Standard error, a& b: Superscripts to be compared statistically within the same raw, Mean Values with different letter superscripts are significantly different ($P<0.05$).

Table (2) Effects of Pseudomonas aeruginosa infection (10^6 colony-forming units (CFUs)/ml) on serum interleukin-9 (IL-9) concentrations in male rabbits

Parameter	control	Weeks post-infection by pseudomonas		
		2 weeks	4 weeks	8weeks
IL-9 (pg/ml)	12.33 ± 4.31^{a}	50.77 ± 8.71^{b}	38.83 ± 2.67^{b}	19.01 ± 5.37^{a}

Data are presented as (Mean ± S.E), S.E = Standard error, a& b: Superscripts to be compared statistically within the same raw, Mean Values with different letter superscripts are significantly different ($P<0.05$).

Table (3) Effects of Pseudomonas aeruginosa infection (10^6 colony-forming units (CFUs)/ml) on serum tumor necrosis factors (TNF) concentrations in male rabbits

Parameter	control	Weeks post-infection by pseudomonas		
		2 weeks	4 weeks	8weeks
TNF-α (pg/ml)	16.10 ± 2.35^{a}	55.45 ± 7.98^{b}	49.03 ± 7.42^{b}	22.40 ± 4.63^{a}

Data are presented as (Mean ± S.E), S.E = Standard error, a & b: Superscripts to be compared statistically within the same raw, Mean Values with different letter superscripts are significantly different ($P<0.05$).

4. DISCUSSION

Chronic suppurative otitis media (CSOM) and various complications associated with the disease such as irreversible local destruction of middle ear structures, facial palsy, serious intracranial and extracranial complications are among the most common conditions seen by the otologist,

paediatrician and the general practitioner. It is a persistent disease and often causes irreversible local destruction of middle ear (Jang and Park, 2004). As Pseudomonas aeruginosa was the most prevalent microorganism that is also supported by national and international studies (Yeo et al., 2007)

Many studies have reported that the most frequently detected frequently in patients with CSOM are Staphylococcus and Pseudomonas aeruginosa (PA); increases in the resistance of these pathogens to antimicrobial agents have made them difficult to treat (lee et al., 2010). Chronic suppurative otitis media refers primarily to chronic inflammation of the middle ear and mastoid cavity, with a perforated tympanic membrane and drainage. CCOM has been found to occur when keratinized squamous epithelium invades the middle ear and destroys nearby tissues. CCOM may also be involved in the severity and maintenance of inflammation (Semaan and Megerian., 2006). Experimental models of chronic suppurative otitis media (CSOM) are less common in the literature. Pseudomonas aeruginosa is the dominant microorganism causing recurrent suppuration in chronic human ears [6], and one of the most frequent causes of chronic otitis media with cholesteatoma (Kenna and Bluestone., 1986).

Table (1): represented that serum interleukin-2 tends to decrease during the acute stage and tends to increase in the semi chronic infection and there is a significant difference in the second and fourth weeks between control and experimental groups. This results similar to the results obtained by (Melhus and Ryan., 2000) who reported that IL-2 was not detected in middle ear with effusion (MEE) of acute otitis media (AOM) patients by ELISA nor was it found in an experimental rat model of AOM induced by inoculation of Streptococcus pneumoniae type 3 and nontypable H. influenzae by reverse transcription-polymerase chain reaction (RT-PCR). On the contrary, IL-2 was detected in the MEE of the chronic otitis media with effusion (OME) patients by ELISA (Jang and Kim, 2002). (Smirnova et al., 2004) reported that an imbalance in the production of these cytokines Induces switching to the chronic stage. Excessive IL-2 production can cause humoral inflammatory processes and/or chronic cell-mediated processes whereas IL-2 deficiency promotes persistence of OME, which can result in chronic OME. From this, it may be deduced that the ongoing chronic inflammatory state associated with chronic OME may contribute different cell types, and thus different cytokines, than those with AOM.

Table (2): represented that serum interleukin-9 tends to increase during the two and fourth weeks of experimental group compared to the control group and after then tends to decrease levels in the eight weeks. This result similar to the results obtained by (Hebda et al., 2002) who reported that investigations of cytokine profiles in different experimental models of OME showed a rapid appearance of IL-10 in the early stages of acute otitis media. But later studies showed that IL-9 has a weak effect in proliferation of primary T cells, despite the fact that proliferation of certain T-cell clones can be strongly stimulated by IL-9. Instead, IL-9 exhibits other functions, most noticeably in proliferation of mast cells, goblet cells and airway mucin-producing cells. Thus, in many ways, IL-9 is different from other γc cytokines as a T-cell growth factor (Knoops and Renauld, 2004)

Table (3): represented that serum tumor necrosis factor -alpha (TNF-α) tends to increase during the two and fourth weeks of experimental group compared to the control group and after then tends to decrease levels in the eight weeks. This result comes in accordance with (Scharer et al., 2003) reported that the cytokines IL-1_ and TNF-_ appear to be elevated in the serum of patients with *S. pneumoniae* AOM.

Moreover, (Scharer et al., 2003) reported that the mean concentration of all cytokines was elevated at diagnosis of AOM compared to levels in healthy controls, yet only IL-6 reached statistical significance (*P*- 0.05). In contrary, (Lee et al., 2013) reported that in OME with the presence of bacterial pathogens. IL-12, TNF-α, IFN-β, and IL-6 expression tended to decrease with the detection of bacteria. The presence of bacterial pathogens in OME may be related to innate immunity abnormalities

5. CONCLUSION

The present study demonstrated that inflammatory mediators such as (TNF-α) and interleukin-2 play a central role in the pathogenesis of OM and cholesteatoma by initiating and maintaining the inflammatory response to infection and injury. Inflammatory mediators may be one of the reasons that some patients progress from acute to COM and cholesteatoma. We recommended that, future studies are necessary to elucidate the roles of the newly discovered chemokines in the pathogenesis of OM and cholesteatoma.

6. REFERENCES

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