Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6040 - 6054
Received 25 April 2021; Accepted 08 May 2021.

Host Immune Responses to Otopathogens during Otitis Media

Department of Microbiology, College of Medicine, University of Babylon, Republic of Iraq
Authors: Inam Mohammed Abed, Ifad Kerim Al-Shibly, Safaa Sahib Naji Sultan and Safaa Hussain Alturaihy
Author of Correspondence E-mail: ifad_shibly@uobabylon.edu.iq

Abstract

A hundred and twenty individuals were enrolled in this 1:1 case-control study. Whole blood samples were collected from sixty apparently healthy control subjects while ear swabs and whole blood samples were collected from sixty patients with otitis media, who were attended to the outpatient clinic of ENT department in Al-Hilla Teaching Hospital/ Babylon and Al-Imam Al-Sadiq Hospital/ Babylon and from some private clinics during a period extended from October-2020 to January-2021. Patients were diagnosed by otolaryngology consultant surgeon and were examined by otoscope. About patients with otitis media, in the current study the number and proportion of male patients were more than that of female patients, 34 (56.7 %) versus 26 (43.3 %), and the male to female ratio showed slight male predilection, 1.3:1. The difference in the frequency distribution of patients with otitis media according to gender was not significantly different from that of control subjects (p = 0.446).In the current study, that age of onset of otitis media among patients is highly variable and has a wide range; nevertheless, children under 10 years of age were the predominant group affected accounting for 31.7 % out of all patients in the study.Clinically, most of patients in the current study had acute otitis media (AOM) accounting for 76.7 % followed by chronic suppurative otitis media (CSOM) accounting for 15.0 % and lastly by otitis media with effusion (OME) accounting for 8.3 % only. Recurrent inflammation is shown in 19 (31.7 %) of cases.In the current study, the most common blood group was A (36.7 %) followed by B (26.7 %) then by O (23.3 %) and lastly AB (13.3 %). There was no significant difference in the frequency distribution of individuals according to blood group between patients with otitis media and control group (p<0.05). However, there was a highly significant difference in the frequency distribution of individuals according to blood group O between patients with otitis media and control group (p = 0.007). Therefore, according to the current study, none of the blood group was a risk factor, but on the contrary, blood group O was proved as a protective factor against otitis media by approximately 55 %, with an odds ratio of 0.35 (Confidence interval of 0.16 -0.76).The comparison of serum ferritin level between patients with otitis media and control group revealed that ferritin level was lower in patients with otitis media in comparison with control group in a highly significant manner (p< 0.001), in terms of median (inter-quartile range) the levels were 30.43 (49.61) versus 100.30 (67.65), in patients and control groups, respectively. The results of bacterial culture showed the growth of the following microorganisms with corresponding proportions: *Staphylococcus aureus* accounting for 17 (28.3 %); *Staphylococcus epidermidis* accounting for 6 (10.0 %); *Klebsiella pneumoniae* accounting for 9 (15.0 %); *Klebsiella oxytoca* accounting for 2 (3.3 %); *Pseudomonas aeruginosa* accounting for 9 (15.0 %); *Pseudomonas stutzeri* accounting for 2 (3.3 %); *Escherichia coli* accounting for 6 (10.0 %); *Streptococcus pneumoniae* accounting for 3 (5.0 %); *Acinetobacter baumannii* accounting for 3 (5.0 %) and *Candida albicans* accounting for 3 (5.0 %). This study revealed that both gram-positive and gram-negative organisms were responsible for infection of middle-ear. Gram-negative rods outnumber the gram-positive organisms. Otherwise, *Streptococcus pneumoniae* remains the most frequently detected bacteria from patients with AOM in this study.

Keywords: Otitis Media, AOM, CSOM, OME, Human Beta Defensin 2,DEFβ2/DEFB2, Blood group, Ferritin, Bacterial Infection.

Introduction

Otitis media (OM) is media is one of the most common causes of infective and inflammatory conditions affecting the middle ear, with a variety of subtypes

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6040 - 6054
Received 25 April 2021; Accepted 08 May 2021.

differing in presentation, associated complications, and treatment. The most damaging complication of otitis media is temporary or permanent hearing loss is (Monasta *et al.*, 2012; Hardani *et al.*, 2020). Otitis media is pathology of the middle ear and middle ear mucosa, behind the ear drum (tympanic membrane). The middle ear is a cavity containing the ear ossicles (malleus, incus, and stapes), with the eustachian tube placed anteriorly (leading to the nasopharynx), the mastoid air cells posteriorly, tympanic membrane laterally, and the inner ear medially (Luers and Hüttenbrink, 2016).

Otitis media is defined as an infection of the middle ear space. It is a spectrum of diseases that include acute otitis media (AOM), chronic suppurative otitis media (CSOM), and otitis media with effusion (OME). Acute otitis media is the second most common pediatric diagnosis in the emergency department following upper respiratory infections. Although otitis media can occur at any age, it is most commonly seen between the ages of 6 to 24 months. (Danishyar and Ashurst 2020)

Otitis media is diagnosed clinically via objective findings on physical exam (otoscopy) combined with the patient's history and presenting signs and symptoms. Several diagnostic tools are available such as a pneumatic otoscope, tympanometry, and acoustic reflectometry to aid in the diagnosis of otitis media. Pneumatic otoscopy is the most reliable and has a higher sensitivity and specificity as compared to plain otoscopy, though tympanometry and other modalities can facilitate diagnosis if pneumatic otoscopy is unavailable (Lieberthal *et al.*, 2013; Danishyar and Ashurst 2020).

The middle ear epithelial cells primarily release beta defensins, which are cationic proteins with antimicrobial function against a wide range of viruses, bacteria, fungi, and protozoa (Yang *et al.*, 2007; Mittal *et al.*, 2014). Their major antimicrobial mechanism is thought to be through the formation of a pore into the microbial membrane. However, some defensins are known to stimulate pro-inflammatory cytokines/ chemokines, to act as chemoattractants for neutrophils, mast cells, T cells, and dendritic cells, and to directly inhibit bacterial toxins (Yang *et al.*, 2007; Mittal *et al.*, 2014). The up-regulated expression of mouse β-defensins 2, 3, and 4 has been demonstrated in the tubotympanums in experimental OM, while no such up-regulation was seen in the middle ears of healthy controls (Jin Shin *et al.*, 2006). Human β-defensin 2 (HBD2) is seen to be up-regulated in the middle ear response to bacteria and cytokines like non-typeable *Haemophilus influenzae* (NTHi) and interleukin (IL)-1α (Moon *et al.*, 2006; Lee *et al.*, 2008).

Methods

Ethical approval involved verbal and written consent were taken from each patient before sampling specimens. This study was approved by the committee on publication ethics at college of medicine, University of Babylon, Iraq, under the reference No. BMS/0235/016.

Subjects of The Study

A case-control study design including 60 samples of otitis media types and blood sera obtained from patients infected with otitis media and the same number of healthy control group, attended to Al-Hilla Teaching Hospital/ Babylon and Al-Imam Al-Sadiq Hospital/ Babylon, during period extended from October of 2020 to January of 2021.Patients were diagnosed by experienced ENT physicians, andexamined by Otoscope. Sterile transport medium swaband whole blood samples were collected. The swabs including inside tubes of transport media were transported in bag contains ice packs and then stored in laboratory at 4 ℃till they were used in microbial cultivation. The blood samples collected in gel and clot activator vacuum tubes which were centrifuged to separate sera at supernatant to be evacuated in Eppendorf tubes for storing in deep freezer at -20 ℃were used for measurement of human beta defensin 2 and ferritin. Whereas, the EDTA tube(anticoagulant) samples were used forblood typing test (ABO).

Inclusion Criteria

Inclusion criteria included the patients were diagnosed by a specialized physician depending on

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6040 - 6054
Received 25 April 2021; Accepted 08 May 2021.

clinical finding and proved by Otoscope.

Exclusion Criteria

Exclusion criteria of OM patients were involved:patients with respiratory system diseases, patients with dental problem e.g., preapical inflammations for posterior teeth, patients with inflammations of parotid gland and submandibular salivary glands and cervical lymph nodes, patients with tonsilitis and pharyngitis.

Identification of Bacterial Isolates

Staining and Microscopic Examination

Gram stain used in microscopic examination to differentiate gram negative from gram positive bacteria and to identify their shape and arrangement.

Identification of Bacterial and Yeast Isolates

The bacterial isolates were diagnosed according to the results report of the automated Vitek 2 system. The procedure by which the prepared the bacterial and yeast suspension was depended to the instructions of Vitek 2 kit (BioMerieux Company/ France).

ELISA Method

ELISA was used in this study for quantitative determination of Human DEFβ2/DEFB2 (Defensin Beta 2) concentrations titer from in serum samples and done according to company instruction (Elabscience/ Spain) as the following assay procedure

Blood Typing Test

The blood typing test (ABO) using ABO set. The procedure by which the prepared the bacterial and done according to company instructions.

Results and Discussion

Disease Characteristics of Patients with Otitis Media

The Frequency Distribution of Patients with Otitis Media According to Mode of Clinical Presentation

The frequency distribution of patients with otitis media according to mode of clinical presentation is shown in table (1) and figure (1). Most of patients in the current study had acute otitis media (AOM) accounting for 76.7 % followed by chronic suppurative otitis media (CSOM) accounting for 15.0 % and lastly by otitis media with effusion (OME) accounting for 8.3 % only.

Table (1): The frequency distribution of patients with otitis media according to mode of clinical presentation.

Type of Inflammation	Number of cases	%
AOM	46	76.7
CSOM	9	15.0
OME	5	8.3

AOM: acute otitis media; **OME**: otitis media with effusion; **CSOM**: chronic suppurative otitis media.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6040 - 6054
Received 25 April 2021; Accepted 08 May 2021.

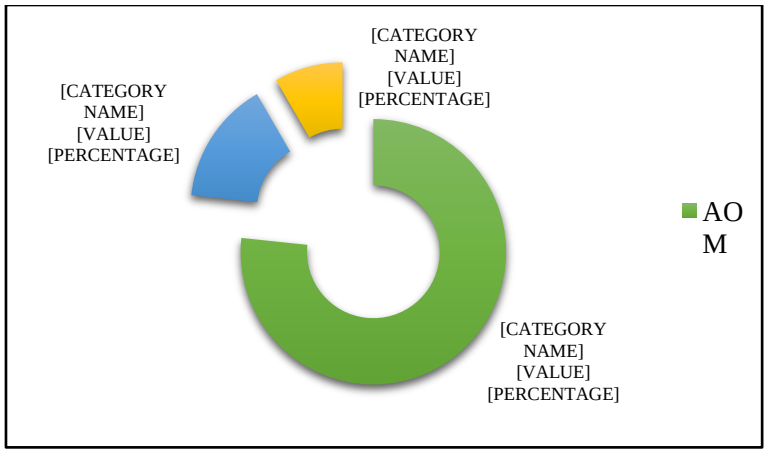

Figure (1): Exploded doughnut graph showing the frequency distribution of patients with otitis media according to mode of clinical presentation. **AOM**: acute otitis media; **OME**: otitis media with effusion; **CSOM**: chronic suppurative otitis media.

The inflammation of otitis media is categorized according to severity of clinical presentation, duration of disease and associated clinical manifestations into acute otitis media (AOM), Otitis media with effusion (OME), chronic otitis media (COM) and chronic suppurative otitis media (CSOM). Acute otitis media is characterized by acute inflammation of middles ear up on clinical examination whereas otitis media with effusion is characterized by effusion with lack of clear evidence of acute infection. Chronic otitis media is characterized by existence of such effusion for more than 3 months (Gotcsik, 2012). Chronic suppurative otitis media is characterized by discharge from middle ear through a perforated tympanic membrane (Gotcsik, 2012).

It has been shown according to a systemic review done on 2012 that the annual incidence rate of acute otitis media is estimated at 10.8 new cases per 100 persons (Monasta, 2012). There is great variation in this annual incidence rate throughout the world with as low as 3.6 % in central Europe to as high as 43.4 % in some African countries. The incidence rate is higher in children and the estimate of acute otitis media at that age may reach 51 % (Monasta, 2012). The higher incidence of acute otitis media in above literatures is in line with the high rate of acute otitis media in the current study.

Regarding CSOM, the global the annual incidence rate in average is estimated to be 4.8 new cases per 1,000 people including all ages. It has been shown that around 22 % of children less than 5 years are affected by new cases of CSOM (Monasta, 2012). These estimates support the findings of the current study in that the rate of CSOM was 15 %.

There generally great difficulty in the estimation of the true incidence or prevalence rate of otitis media with effusion because it is usually asymptomatic however previous estimates according to large cohort studies have shown that OME accounts for 20 % and that children are mostly affected (Schilder *et al.*, 2016). The low rate of OME globally is therefore in line with the low rate of OME seen in the present study and the reason is the same as most patients are asymptomatic and do not seek medical advice.

The Rate of Recurrence Inflammation Reported in The Current Study in Patients with Otitis Media

The rate of recurrence inflammation reported in the current study in patients with otitis media is shown in table (2) and figure (2). Recurrent inflammation is shown in 19 (31.7 %) of cases. None of patients enrolled in the current study had chronic medical illness.

Recurrent inflammation was seen 31.7 % of our enrolled patients. Regarding recurrent inflammation, the incidence rate in a previous report was estimated to average between 20 and 30 %.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6040 - 6054
Received 25 April 2021; Accepted 08 May 2021.

Recurrence is sometimes seen within less than one month of completing anti-bacterial therapy due to failure of this treatment. The clinical distinction of recurrent inflammation form otitis media with effusion is essential because recurrent inflammation is sometimes associated with risk of hearing loss (Pichichero, 2000).

Chronic medical illness was not seen in any of our patients. In previous reports, it has been shown that chronic otitis media is associated with chronic medical illness such as diabetes mellitus, systemic hypertension, pulmonary tuberculosis and chronic rhinosinusitis (Park *et al.*,2015). Some reports have shown that a number of cardiovascular disorders and diabetes are among risk factors of ear infection (Ito *et al.*, 2007; Austin *et al.*, 2009; Kakarlapudi *et al.*, 2009; Bainbridge *et al.*, 2010).

The high prevalence of chronic otitis media among patients with patients with chronic medical illnesses is attributed to problems with the immune system because these medical illnesses are associated with protein energy malnutrition and disturbance of innate and adaptive immune response (Holtzman, 2012; McLaughlin *et al.*, 2017; Bagatini *et al.*, 2018).

Table (2): The rate of recurrence inflammation reported in the current study in patients with otitis media.

Recurrent Inflammation	Number of Cases	%
Positive	19	31.7
Negative	41	68.3

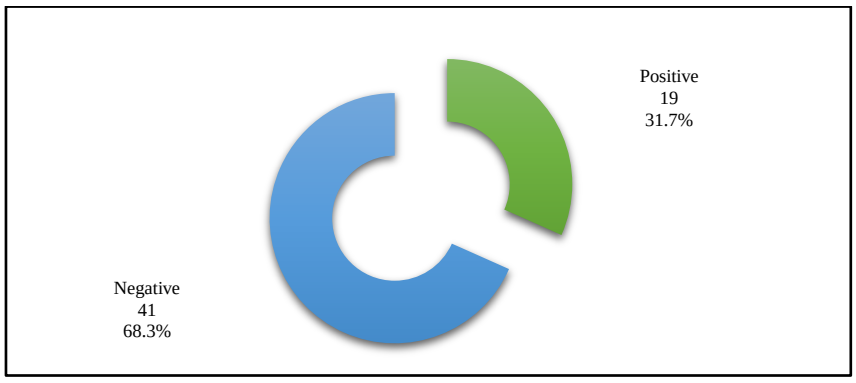

Figure (2): Exploded doughnut graph showing the rate of recurrence inflammation reported in the current study in patients with otitis media.

Frequency Distribution of Patients and Control Subjects According to Blood Group

The frequency distribution of patients and control subjects according to blood group is shown in table (3) and figure (3). In the current study, the most common blood group was A (36.7 %) followed by B (26.7 %) then by O (23.3 %) and lastly AB (13.3 %). There was no significant difference in the frequency distribution of individuals according to blood group A between patients with otitis media and control group ($p = 0.330$). There was also no significant difference in the frequency distribution of individuals according to blood group B between patients with otitis media and control group ($p = 0.068$). In addition, there was no significant difference in the frequency distribution of individuals according to

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6040 - 6054
Received 25 April 2021; Accepted 08 May 2021.

blood group AB between patients with otitis media and control group (p = 1.000). However, there was highly significant difference in the frequency distribution of individuals according to blood group O between patients with otitis media and control group (p = 0.007) in such a way that blood group blood group O was less frequent in patients with otitis media in comparison with control group, 14 (23.3 %) versus 16 (45.7 %), respectively. Therefore, according to the current study, none of the blood group was a risk factor, but on the contrary, blood group O was proved as a protective factor with an odds ratio of 0.35 (Confidence interval of 0.16 -0.76). Thus, blood group O provides protection against otitis media by approximately 55 %.

Table (3): Frequency distribution of patients and control subjects according to blood group.

Blood Group	Control n = 60	Patients n = 60	p	OR (95% CI)
Blood Group A, *n* (%)	17 (28.3 %)	22 (36.7 %)	0.330 C NS	1.46 (0.68 -3.16)
Blood Group B, *n* (%)	8 (13.3 %)	16 (26.7 %)	0.068 C NS	2.36 (0.92 -6.04)
Blood Group AB, *n* (%)	7 (11.7 %)	8 (13.3 %)	1.000 Y NS	1.16 (0.39 -3.44)
Blood Group O, *n* (%)	28 (46.7 %)	14 (23.3 %)	0.007 C HS	0.35 (0.16 -0.76)

C: Chi-square test; **Y**: Yates correction for continuity; **NS**: not significant at $p > 0.05$; HS: highly significant at $p \leq 0.01$

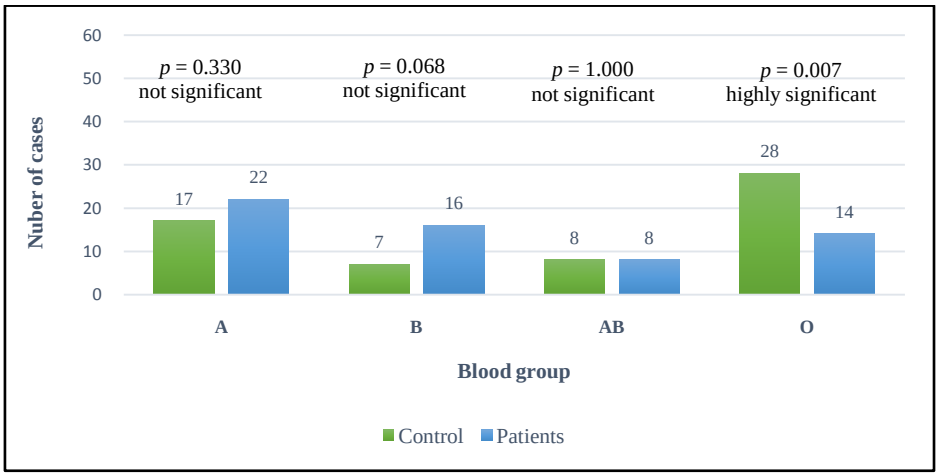

Figure (3): Clustered cylinder graph showing the frequency distribution of patients and control subjects according to blood group.

Indeed, the present study finding of the protective role of blood group O is in line with the study of Apostolopoulos et al in 2002 who found that blood group O was associated significantly with lower risk of otitis media in comparison with other groups. On the other hand, Apostolopoulos et al in 2002 found no significant association with otitis media and this finding is in agreement with the findings of the current study.

In another study done in 2019 by Wiesen *et al*, blood group O was found to be protective against otitis media while blood group A was a risk factor. Actually, the current study agrees with Wiesen et al, in 2019 in that blood group O is a risk factor; however, in the current study, the blood group A was more

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6040 - 6054
Received 25 April 2021; Accepted 08 May 2021.

frequent in patients with otitis media in comparison with control group, 22 (36.7 %) versus 10 (28.6 %), respectively, but the difference did not reach statistical significance ($p = 0.421$).

The most possible explanation for the protective role of blood group O is the immunity provided by antibodies circulating in the blood of individuals with blood group O against viral upper respiratory tract infection and the low rate of upper respiratory infection is going to be associated with lower risk of otitis media.

Comparison of Serum Ferritin Level Between Patients with Otitis Media and Control Group

The comparison of serum ferritin level between patients with otitis media and control group is shown in table (4) and figure (4). In the comparison of serum ferritin level between patients with otitis media and control group revealed that ferritin level was lower in patients with otitis media in comparison with control group in a highly significant manner ($p< 0.001$), in terms of median (inter-quartile range) the levels were 30.43 (49.61) versus 100.30 (67.65), in patients and control groups, respectively.

Table (4): Comparison of serum ferritin level between patients with otitis media and control subjects

Serum ferritin (ng/ml)	**Control group *n* = 60**	**Patient group *n* = 60**
Median	100.30	30.43
IQR	67.65	49.61
Minimal level	1.35	4.90
Maximum level	198.01	174.50
p-value	< 0.001 M HS	

n: number of cases; **IQR**: inter-quartile range; **M**: Mann Whitney U test; **HS**: highly significant at $p \leq 0.01$

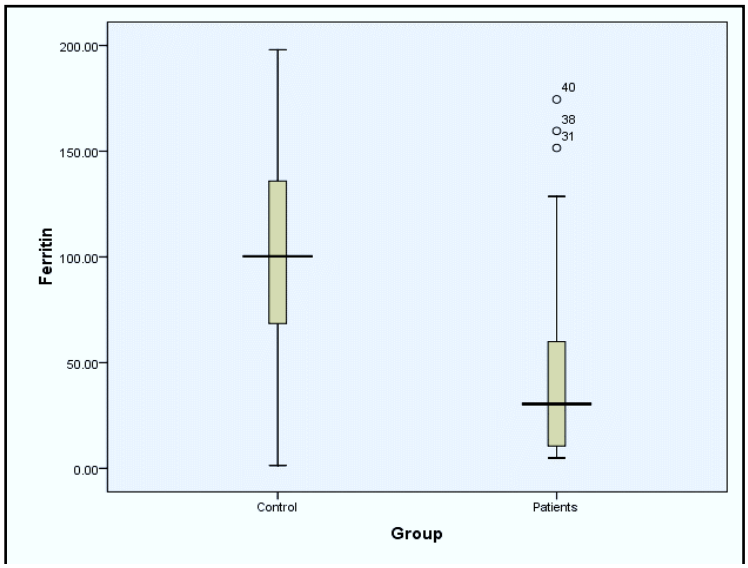

Figure (4): Box plot showing comparison of serum ferritin level between patients with otitis media and control subjects.

The current study finding is in agreement with the finding of Schieffer et al (2017) who observed significant association between low serum ferritin and otitis media. Low serum ferritin is an indication of

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6040 - 6054
Received 25 April 2021; Accepted 08 May 2021.

iron deficiency anemia (Schieffer *et al.,* 2017), thus, the finding of the current study suggests that iron deficiency anemia is a significant predisposing factor for otitis media. The current observation of significant association between iron deficiency and anemia and otitis media is in line with previous observation of several authors (Akcan *et al.,* 2019; Jayaweera, *et al.,* 2019; Schieffer *et al.,* 2017).

Indeed, our results in the current study are in line with the results of Akcan *et al.*, in 2019. Akcan *et al*., in 2019 examined the association between low serum ferritin and otitis media with effusion and they found significant association and the difference in mean serum ferritin was highly significant ($p<$ 0.001). Akcan *et al.,* in 2019 linked this association to iron deficiency anemia.

There is growing bulk of evidences that iron deficiency anemia may raise susceptibility to infections in child age group as well as in adult population. This susceptibility could be attributed to impaired macrophage and neutrophil function and reduced pro-inflammatory cytokines macrophage (Oppenheimer, 2002; Wintergerst *et al.,* 2007). An important role is offered by iron in pathways of DNA replication related to immunoprogenitor cells. Research work has shown that iron insufficiency is more frequent in individuals who have repeated infections of upper respiratory tract (Muñoz *et al.,* 2007). Thus, children and adults with repeated upper respiratory tract infection are more prone to otitis media since this will predispose to inflammation of Eustachian tube and secondary involvement of middle ear.

After entrance of microorganism to the organ vicinity a number of factors concurrently participate for the establishment of the infection. When considering host-microorganism interface, nutritional status of the individual is one of the important contributory factors for invasion and establishment of infections (Wang *et al.*,2014). Hemoglobin (Hb) concentration is an index that reflects the long-term nutritional status and also blood oxygen carrying ability. Children are prone to a rapid growth state; therefore, they demand enormous nutrition. In addition, the tendency to develop malnutrition is also high. In such cases, the risk for occurrence of infection is high and the vicious cycle works to cause poor-nutrition (Jayaweera *et al.,* 2019).

Anemia is affecting approximately one third of children all over the world. A number of factors are contributed to anemia. In childhood period, nutritional anemia including vitamin B-12, iron and folate deficiency is the most prevalent (Levin *et al.,* 2016). Moreover, hereditary type of anemia including sickle cell anemia and thalassemia are seen (Kotecha, 2011). Irrespective of the cause following anemia, child complain of fatigability and its negative effect on growth is big. Iron deficiency anemia in children happens most commonly between the age of 6 months and 3 years, the period of age when repeated infections happen. Anemia associated recurrent respiratory tract infections occurs more frequently in children than in adults (Jayaweera *et al.,* 2019).

Recurrent infective episodes invariably lead to undernutrition (Tewary, and Narchi, 2015). Probably, anemia is a well-known risk factor for recurrent infective attacks. Moreover, studies on hemoglobin level and development of multiple episodes of otitis media in children have been considered (Jayaweera *et al.,* 2019).

Comparison of Defensin Beta 2 Level Between Patients with Otitis Media and Control Group

The comparison of defensin beta 2 level between patients with otitis media and control group is shown in table (5) and figure (5). In the current study, comparison of defensin beta 2 level between patients with otitis media and control group revealed that the level was higher in patients' group in a highly significant manner ($p<$ 0.001), in terms of median (inter-quartile range) the levels were 3053.90 (2256.94) versus 411.69 (573.75), in patients and control groups, respectively.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6040 - 6054
Received 25 April 2021; Accepted 08 May 2021.

Table (5): Comparison of defensin beta 2 level between patients with otitis media and control group.

Serum Defensin Beta 2	Control Group ***n* = 60**	Patient Group ***n*= 60**
Median	395.91	3053.9
IQR	354.65	2256.94
Minimal Level	49.62	537.95
Maximum Level	1581.48	4697.19
P-Value	< 0.001 M HS	

n: number of cases; **IQR**: inter-quartile range; **M**: Mann Whitney U test; **HS**: highly significant at $p \leq 0.01$.

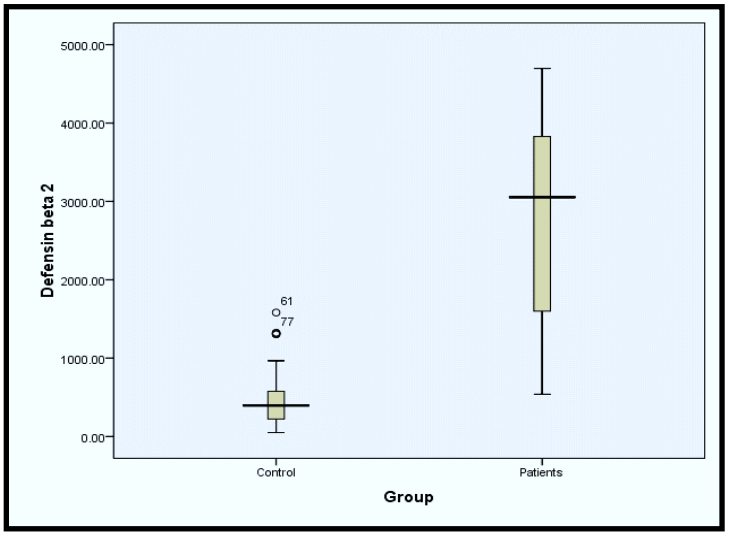

Figure (5): Box plot showing the comparison of defensin beta 2 level between patients with otitis media and control group.

In one previous experimental study, the expression of β defensin 2 was shown to be unregulated in association with otitis media (Jin Shin *et al.*, 2006) and this observation supports the higher level of β defensin 2 found in the present study in comparison with control group and suggests an important role for this molecule in otitis media. In one recent study, the level of β defensin 2 was estimated in the effusion fluid of middle ear in patients with otitis media and found to be higher in patients whohas positive culture for bacteria than those with negative culture results; however, the difference was not statistically significant ($p > 0.05$) (Seppanen *et al.*, 2019). It should be noted that in Seppanen et al study, both groups were otitis media patients while in current study, comparison was made between control group and patients with otitis media. An experimental study has shown that β-defensin 2 possess a strong anti-viral activity against viral infections of respiratory tract (Gong *et al.*, 2010).

Actually, flowing thorough search in the available articles, we did not find a clinical study comparing β defensin 2 between patients with otitis media and healthy control subjects, therefore, this point can be considered as an originality for our study. Indeed, it has been stated that in both human middle ear epithelial cell culture and animal otitis media models, β-defensins are highly expressed and efficiently kill the usual pathogens associated with otitis media (Underwood and Bakaletz, 2011).

Defensins are peptides that possess broad-spectrum antimicrobial activity. They play an important role in the innate immune system, offering fast protection against pathogens and limiting the microbiota of several important anatomic sites in the body (Salzman et al., 2010; Salzman, 2007). In humans, β-

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6040 - 6054
Received 25 April 2021; Accepted 08 May 2021.

defensins are produced by epithelial cells in the upper and lower airway (including the middle ear) (Underwood and Bakaletz, 2011).

Otitis media (OM), one of the most common disorders in young children, is clinically essential owing to its high prevalence in children and its potential effect on motor coordination and language development. OM is the most frequent reason for the giving of antibiotics (accounting for quarter of prescriptions) due to its extremely high prevalence. An increase in antibiotic resistance among OM microorganisms is emerging as a major public health issue worldwide, which led health workers to consider non-antibiotic approaches for the treatment of it. In one study, Woo *et al* in 2015 evaluated "gene transfer of an antimicrobial peptide, human β-defensin 2 (DEFB4), using an adenoviral vector (Ad5 with deletions of E1/E3/E4) as a potential therapeutic approach". They showed that the transduction of human β-defensin 2 induces the production of human β-defensin 2 and suppresses non-typeable *Haemophilus influenzae* (NTHi) adhesion to human middle ear epithelial cells. In addition, intratympanic inoculation of Ad-DEFB4 was shown to minimize NTHi-induced middle ear effusions with no associated significant immune reaction. Of great concern, intratympanic inoculation of Ad-DEFB4 was shown to significantly potentiate clearance of NTHi from cavity of middle ear. In summary, Woo *et a*l results suppose that intratympanic gene delivery of antimicrobial molecules can serve as an adjuvant way for the treatment of OM (Woo *et al.*, 2015).

In previous study, serum DEF-β2 was studied as immune parameter for diabetic patients, the results showed that the DEF-β2 level in the diabetes group was, high in the infection (Al-Shibly *et al.*, 2019).

Results of Culture Obtained from Patients with Otitis Media Enrolled in The Present Study

The results of culture obtained from patients with otitis media enrolled in the present study are shown in table (6). In the current study, results of growth on culture media that confirmed by Vitek 2 system showed the of the following microorganisms with corresponding proportions: *Staphylococcus aureus* accounting for 17 (28.3 %); *Staphylococcus epidermidis* accounting for 6 (10.0 %); *Klebsiella pneumoniae* accounting for 9 (15.0 %); *Klebsiella oxytoca* accounting for 2 (3.3 %); *Pseudomonas aeruginosa* accounting for 9 (15.0 %); *Pseudomonas stutzeri* accounting for 2 (3.3 %); *Escherichia coli* accounting for 6 (10.0 %); *Streptococcus pneumoniae* accounting for 3 (5.0 %); *Acinetobacter baumannii* accounting for 3 (5.0 %) and *Candida albicans* accounting for 3 (5.0 %).

Corresponding figures reported by other authors vary significantly. Aslam, *et al.*, from Pakistan (2004) in their study on 142 samples revealed that 76% of them were pure and 23.9% were mixed cultures and only 2.1% fungi, whereas, Poorey and lyer from India (2002) in their study on 100 samples found pure growth from 82, mixed growth from 10, and no growth in 8 samples.

Table (6): Results of culture obtained from patients with otitis media enrolled in the present study.

Microorganism	Number of Isolates	%
Staphylococcus aureus	17	28.3
Staphylococcus epidermidis	6	10.0
Klebsiella pneumoniae	9	15.0
Klebsiella oxytoca	2	3.3
Pseudomonas aeruginosa	9	15.0
Pseudomonas stutzeri	2	3.3
Escherichia coli	6	10.0
Streptococcus pneumoniae	3	5.0
Acinetobacter baumannii	3	5.0
Candida albicans	3	5.0

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6040 - 6054
Received 25 April 2021; Accepted 08 May 2021.

Difference in results of various authors could have been due to the difference in the patient population studied and geographical variations. In the present study, mono-microbial etiology was found to be more common (51.84%) and this observation was supported by other researchers (Loy *et al.*, 2002; Shyamla and Reddy, 2012). A study from Iran (Ettehad *et al.,* 2006) reported mono-microbial etiology to be 100% in all 61 samples studied.

Predominant bacterial etiology (aerobic) of OM in this region is *Staphylococcus aureus* and this observation was in line with diversity of microbial flora of OM infection in other regions as reported in studies by Ettehad, *et al.*, (2006) from Iran (31.15%) and Singh, *et al.*, (2012) from India (36%). In contrast, other studies from India, (Kumar and Seth, 2011) Nigeria, (Osazuwa *et al.,* 2011) and Pakistan (Mansoor *et al.*, 2009) showed different trends as *Pseudomonas* was the most prevalent organism and this could be due to the variation in microorganisms in different regions and effect of climate. In our study, we could isolate *Pseudomonas* in 18.3 % of cases. *Pseudomonas*, however, is the predominant cause of OM in tropical region does not usually inhabit the upper respiratory tract, its presence in the middle-ear cannot be ascribed to an invasion through ET and it should be considered as secondary invader gaining access to the middle-ear via defect in TM (Vishvanath *et al.,* 2012).

In one previous Iraqi study, the *Pseudomonas aeruginosa* was the most frequent species that causes CSOM then *Staphylococcus aureus* and *Proteus mirabilis* (Almedeny, 2012), whereas in our study the *Staphylococcus aureus* was most common isolated bacterium followed by *Pseudomonas aeruginosa.*

Coliforms including *Klebsiella pneumoniae* and *Escherichia coli* were isolated also, and these findings were tandem to the reports by Mansoor, *et al.* (2009) who reported the same to be 8% and 4% whereas Poorey and lyer (2002) reported a high-incidence for *Klebsiella* in their study (25.4%). A study by Shyamala and Reddy from India (2012) showed a little different trend where *E. coli* was reported in 12% and *Klebsiella* in 5% of cases. More frequent isolation of fecal bacteria like *E. coli*, *Klebsiella* and water bacteria like *Pseudomonas* indicates that individuals are at high-risk of infection due to poor hygiene conditions.

Our study revealed that both gram-positive and gram-negative organisms are responsible for infection of middle-ear. It is usually seen that gram-negative rods outnumber the gram-positive organisms in OM as reported by various authors (Mansoor, *et al.*, 2009; Shyamla and Reddy, 2012). However, in our study *S. aureus* was the predominant organism followed by *Pseudomonas* and other gram-negative rods and this observation corroborates well with reports by other researchers (Singh *et al.*, 2012; Chang *et al.*, 2011).

Fungal infections of the middle-ear are common as fungi thrive well in moist pus. The most commonly found fungi in OM are *Candida* species and *Aspergillus* species (Ibekwe *et al.*, 1997). In the present study, 3 (5.0 %) had fungal growth in the form of *Candida Albicans*. In a study from Haryana, India fungal etiology was found in 15% of cases, out of which 60% were Candida species and 40% were *Aspergillus* species (Kumar and Seth, 2011). In another study from Singapore (Osazuwa *et al.,* 2011) on 90 patients of otitis media, fungi accounted for 8.8% of the total isolates out of which *Aspergillus* species was found in 33.3% followed by *Candida* species 22.2%. These findings may be attributed to the environmental effects on the cases of otitis media, which were studied in this area.

Ngo *et al* in 2016 performed a systemic review about the microbiology in association with otitis media and they found that that *Streptococcus pneumoniae* and *Haemophilus influenzae*, remain the predominant bacterial pathogens, with *S. pneumoniae* the predominant bacterium in the majority reports from AOM patients and these findings support the findings of our study. In contrast, they found that *H. influenzae* was the predominant bacterium for patients experiencing chronic OME, recurrent AOM and AOM with treatment failure.

Streptococcus pneumoniae remains the most frequently detected bacteria from patients with AOM in Asia, Africa, America and Europe. In contrast to AOM, *H. influenzae* is most frequently detected

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6040 - 6054
Received 25 April 2021; Accepted 08 May 2021.

bacteria in the MEF of patients with RAOM or OME/ COME globally and, with the exception of Australia, is the second most common pathogen in patients with AOM across the world.

In Australia, *H. influenzae* is the predominant bacterium for AOM and OME for indigenous and non-indigenous children. Bilateral AOM, eye symptoms, previous treatment with antibiotics, protracted and recurrent disease, are more likely for AOM caused by *H. influenzae* when compared with caused by other pathogens (Ngo *et al.*, 2016)

Conclusions

1. None of the blood group was a risk factor, on the contrary, blood group O was proved as a protective factor against otitis media by approximately 55 %.
2. Serum ferritin level was lower in patients with otitis media in comparison with control group in a highly significant manner.
3. Serum defensin beta 2 level was higher in patients with otitis media than control in a highly significant manner.
4. Both gram-positive and gram-negative organisms are responsible for infection of middle-ear. It is usually seen that gram-negative rods outnumber the gram-positive organisms.

Recommendations

1. Further studies to investigate the role of viral infections in developing of otitis media are recommended.
2. Appropriate and early diagnosis with optimal management for the pediatric patients under 15 years of age with acute otitis media to reduce the risk of conversion to recurrent or chronic disease.
3. Optimize the level of serum ferritin among iron deficiency anemia patients with otitis media.

Acknowledgements

We are extremely thankful to the Collage of Medicine, University of Babylon for providing all requirements and support, which are essential for successful completion of this work.

References

1. Akcan, F. A., Dündar, Y., Bayram Akcan, H., Cebeci, D., Sungur, M. A., & Ünlü, İ. (2019). The Association between Iron Deficiency and Otitis Media with Effusion. The journal of international advanced otology, 15(1), 18–21.
2. Ahmed, M. M. E. (2021). Car-T Cell Therapy: Current Advances and Future Research Possibilities. Journal of Scientific Research in Medical and Biological Sciences, 2(2), 86-116. https://doi.org/10.47631/jsrmbs.v2i2.234
3. Almedeny SA. (2012). Evaluation of the spectrum of bacteria encountered in patients with chronic suppurative otitis media with their antibiotic sensitivity pattern. Kufa Journal for Nursing Science. 2 (3); 1-10.
4. Al-Shibly, I. K., Alhamdany, M. H., Al-Kaif, R. A., Al-Kaif, L. A. Indian Journal of Public Health Research & Development, October (2019), Vol.10, No. 10
5. Apostolopoulos K, Labreopoulou E, Konstantinos B, *et al*. (2002) Blood group in otitis media with effusion. ORL J Otorhinolaryngol Relat Spec 64:433–435.
6. Aslam MA, Ahmed Z, Azim R. Microbiology and drug sensitivity patterns of chronic suppurative otitis media. J Coll Physicians Surg Pak. 2004;14:459–61.
7. Austin DF, Konrad-Martin D, Griest S, McMillan GP, McDermott D, Fausti S. Diabetes-related changes in hearing. Laryngoscope. 2009;119: 1788–1796.
8. Austin DF, Konrad-Martin D, Griest S, McMillan GP, McDermott D, Fausti S. Diabetes-related changes in hearing. Laryngoscope. 2009;119: 1788–1796.
9. Bagatini, M. D., Cardoso, A. M., Reschke, C. R., & Carvalho, F. B. (2018). Immune System and

Chronic Diseases 2018. Journal of immunology research, 2018, 8653572.
10. Bainbridge KE, Cheng YJ, Cowie CC. Potential mediators of diabetes-related hearing impairment in the U.S. population: National Health and Nutrition Examination Survey 1999–2004. Diabetes Care. 2010;33: 811–816.
11. Bainbridge KE, Cheng YJ, Cowie CC. Potential mediators of diabetes-related hearing impairment in the U.S. population: National Health and Nutrition Examination Survey 1999–2004. Diabetes Care. 2010;33: 811–816.
12. Chang J, Lee SH, Choi J, Im GJ, Jung HH. Nasopharynx as a microbiologic reservoir in chronic suppurative otitis media: Preliminary study. Clin Exp Otorhinolaryngol. 2011;4:122–5.
13. Danishyar, A., & Ashurst, J. V. (2020). Acute Otitis Media. In StatPearls. StatPearls Publishing
14. Ettehad GH, Refahi S, Nemmati A, Pirzadeh A, Daryani A. Microbial and antimicrobial susceptibility patterns from patients with chronic otitis media in Ardebil. Int J Trop Med. 2006;1:62–5.
15. Gong T, Jiang Y, Wang Y, Yang D, Li W, Zhang Q, Feng W, Wang B, Jiang Z, Li M. Recombinant mouse beta-defensin 2 inhibits infection by influenza A virus by blocking its entry. Arch Virol. 2010;155:491–498.
16. Gotcsik M. (2012). Otitis Media. Textbook of Clinical Pediatrics, 863–871.
17. Hardani, A. K., Sr, Moghimi Esfandabadi, F., Delphi, M., Ali Samir, M., & Zamiri Abdollahi, F. (2020). Risk Factors for Otitis Media in Children Referred to Abuzar Hospital in Ahvaz: A Case-Control Study. Cureus, 12(8), e9766.
18. Holtzman M. J. (2012). Asthma as a chronic disease of the innate and adaptive immune systems responding to viruses and allergens. The Journal of clinical investigation, 122(8), 2741–2748.
19. Ibekwe AO, al Shareef Z, Benayam A. Anaerobes and fungi in chronic suppurative otitis media. Ann Otol Rhinol Laryngol. 1997;106:649–52.
20. Ito K, Naito R, Murofushi T, Iguchi R. Questionnaire and interview in screening for hearing impairment in adults. Acta Otolaryngol Suppl. 2007; 24–28.
21. Jayaweera JAAS, Reyes M, Joseph A. Childhood iron deficiency anemia leads to recurrent respiratory tract infections and gastroenteritis. Sci Rep. 2019 Sep 2;9(1):12637.
22. Jayaweera, J., Reyes, M., & Joseph, A. (2019). Childhood iron deficiency anemia leads to recurrent respiratory tract infections and gastroenteritis. Scientific reports, 9(1), 12637.
23. Jin Shin D, Gan-Undram S, Jin Kim S, Joon Jun Y, Jung Im G, Hyun Jung H. Expression of beta-defensins in the tubotympanum of experimental otitis media. Acta Otolaryngol. 2006;126:1040–5.
24. Kakarlapudi V, Sawyer R, Staecker H. The effect of diabetes on sensorineural hearing loss. Otol Neurotol. 2003;24: 382–386.
25. Kotecha, P. V. Nutritional Anemia in Young Children with Focus on Asia and India. Indian J Community Med. 36(1), 8–16.
26. Kumar H, Seth S. Bacterial and fungal study of 100 cases of chronic suppurative otitis media. J Clin Diagn Res. 2011;5:1224–7.
27. Lee HY, Takeshita T, Shimada J, Akopyan A, Woo JI, Pan H, *et al*. Induction of beta defensin 2 by NTHi requires TLR2 mediated MyD88 and IRAK-TRAF6-p38MAPK signaling pathway in human middle ear epithelial cells. BMC Infect Dis. 2008;8:87.
28. Levin, C. *et al*. Iron Deficiency and Iron-deficiency Anemia in Toddlers Ages 18 to 36 Months: A Prospective Study. J Pediatr Hematol Oncol 38(3), 205–9 (2016).
29. Lieberthal AS. The diagnosis and management of acute otitis media. Pediatrics. 2013;131:e964–e999.
30. Loy AH, Tan AL, Lu PK. Microbiology of chronic suppurative otitis media in Singapore. Singapore Med J. 2002;43:296–9.
31. Luers, J. C., & Hüttenbrink, K. B. (2016). Surgical anatomy and pathology of the middle ear. Journal of anatomy, 228(2), 338–353.
32. Mansoor T, Musani MA, Khalid G, Kamal M. Pseudomonas aeruginosa in chronic suppurative

otitis media: Sensitivity spectrum against various antibiotics in Karachi. J Ayub Med Coll Abbottabad. 2009;21:120–3.
33. McLaughlin, T., Ackerman, S. E., Shen, L., & Engleman, E. (2017). Role of innate and adaptive immunity in obesity-associated metabolic disease. The Journal of clinical investigation, 127(1), 5–13.
34. Mittal R, Robalino G, Gerring R, Chan B, Yan D, Grati M, Liu XZ. Immunity genes and susceptibility to otitis media: a comprehensive review. J Genet Genomics. 2014 Nov 20;41(11):567-81.
35. Monasta L, Ronfani L, Marchetti F, et al. Burden of disease caused by otitis media: systematic review and global estimates. PLoS One. 2012;7(4):e36226
36. Moon SK, Lee HY, Pan H, Takeshita T, Park R, Cha K, *et al*. Synergistic effect of interleukin 1 alpha on nontypeable Haemophilus influenzae-induced up-regulation of human beta-defensin 2 in middle ear epithelial cells. BMC Infect Dis. 2006;6:12.
37. Muñoz C, Rios E, Olivos J, Brunser O, Olivares M. Iron, copper and immunocompetence. Br J Nutr. 2007;98:24–28.
38. Ngo, C. C., Massa, H. M., Thornton, R. B., & Cripps, A. W. (2016). Predominant Bacteria Detected from the Middle Ear Fluid of Children Experiencing Otitis Media: A Systematic Review. PloS one, 11(3), e0150949.
39. Oppenheimer SJ. Iron and its relation to immunity and infectious disease. J Nutr. 2001;131:616–33.
40. Osazuwa F, Osazuwa E, Osime C, Igharo EA, Imade PE, Lofor P, *et al*. Etiologic agents of otitis media in Benin city, Nigeria. *N Am J Med Sci*. 2011;3:95–8.
41. Park, M., Lee, J. S., Lee, J. H., Oh, S. H., & Park, M. K. (2015). Prevalence and risk factors of chronic otitis media: the Korean National Health and Nutrition Examination Survey 2010-2012. PloS one, 10(5), e0125905.
42. Pichichero ME. Recurrent and persistent otitis media. Pediatr Infect Dis J. 2000 Sep;19(9):911-6.
43. Poorey VK, Lyer A. Study of bacterial flora in csom and its clinical significance. Indian J Otolaryngol Head Neck Surg. 2002;54:91–5.
44. Salzman NH, Hung K, Haribhai D, Chu H, Karlsson-Sjöberg J, Amir E, Teggatz P, Barman M, Hayward M, Eastwood D, Stoel M, Zhou Y, Sodergren E, Weinstock GM, Bevins CL, Williams CB, Bos NA. Enteric defensins are essential regulators of intestinal microbial ecology. Nat Immunol. 2010 Jan;11(1):76-83.
45. Salzman NH, Underwood MA, Bevins CL. Paneth cells, defensins, and the commensal microbiota: a hypothesis on intimate interplay at the intestinal mucosa. Semin Immunol. 2007 Apr;19(2):70-83.
46. Schieffer, K. M., Connor, J. R., Pawelczyk, J. A., & Sekhar, D. L. (2017). The Relationship Between Iron Deficiency Anemia and Sensorineural Hearing Loss in the Pediatric and Adolescent Population. American journal of audiology, 26(2), 155–162.
47. Schilder, A. G., Chonmaitree, T., Cripps, A. W., Rosenfeld, R. M., Casselbrant, M. L., Haggard, M. P., & Venekamp, R. P. (2016). Otitis media. Nature reviews. Disease primers, 2(1), 16063.
48. Seppanen EJ, Thornton RB, Corscadden KJ, Granland CM, Hibbert J, Fuery A, *et al*. (2019) High concentrations of middle ear antimicrobial peptides and proteins and proinflammatory cytokines are associated with detection of middle ear pathogens in children with recurrent acute otitis media. PLoS ONE 14(12): e0227080.
49. Shyamla R, Reddy SP. The study of bacteriological agents of chronic suppurative otitis media–aerobic culture and evaluation. J Microbiol Biotechnol Res. 2012;2:152–62.
50. Singh AH, Basu R, Venkatesh A. Aerobic bacteriology of chronic suppurative otitis media in Rajahmundry. Biol Med. 2012;4:73–9.
51. Tewary, K. & Narchi, H. Recurrent urinary tract infections in children: Preventive interventions other than prophylactic antibiotics. World J Methodol. 26, 5(2), 13–19.
52. Underwood, M., & Bakaletz, L. (2011). Innate immunity and the role of defensins in otitis media.

Current allergy and asthma reports, 11(6), 499–507. https://doi.org/10.1007/s11882-011-0223-6 Woo JI, Kil SH, Brough DE, Lee YJ, Lim DJ, Moon SK. Therapeutic potential of adenovirus-mediated delivery of β-defensin 2 for experimental otitis media. Innate Immun. 2015 Feb;21(2):215-24.

53. Underwood, M., & Bakaletz, L. (2011). Innate immunity and the role of defensins in otitis media. *Current allergy and asthma reports*, *11*(6), 499–507.
54. Vishvanath S, Mukhopadhyay C, Prakash R, Pillai S, Pujary K, Pujary P. Chronic suppurative otitis media: Optimizing initial antibiotic therapy in a tertiary care setup. Indian J Otolaryngol Head Neck Surg. 2012;64:285–9.
55. Wang, H., Liddell, C. A. & Coates, M. M. Global, regional, and national levels of neonatal, infant, and under-5 mortality during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 384(9947), 957–979 (2014).
56. Wiesen, B. M., Hafrén, L., Einarsdottir, E., Kere, J., Mattila, P. S., & Santos-Cortez, R. (2019). ABO Genotype and Blood Type Are Associated with Otitis Media. Genetic testing and molecular biomarkers, 23(11), 823–827.
57. Wintergerst ES, Maggini S, Hornig DH. Contribution of selected vitamins and trace elements to immune function. Ann Nutr Metab. 2007;51:301–23.
58. Yang D, Liu ZH, Tewary P, Chen Q, de la Rosa G, Oppenheim JJ. Defensin participation in innate and adaptive immunity. Curr Pharm Des. 2007;13:3131–9.