BIOMARKERS OF SUPPURATIVE OTITIS MEDIA IN CHILDREN ATTENDING SELECTED HEALTH FACILITIES IN IBADAN

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DEDICATION

Salmot Abike Olasunkade

The top hierarchy is never fully occupied but reserved for only those who persevere in endless search for knowledge

CERTIFICATION 1

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ABBREVIATIONS

OM	-	Otitis media
SOM	-	Suppurative otitis media
AOM	-	Acute otitis media
ASOM-		Acute Suppurative Otitis Media
CSOM -		Chronic suppurative otitis media
ESOM-		Early suppurative otitis media
EOM	-	Early otitis media
MES	-	Middle Ear Secretion
Ig	-	Immunoglobulin
IFN-γ -		interferon gamma
TNF α -		Tumour necrosis factor alpha
IL	-	Interleukin
PTA	-	Pure tone average
pg/mL -		Picogram per milliliter
μL	-	Microlitre
μg/L	-	Microgram per litre
Zn	-	Zinc
WHO	-	World Health Organization
AAOHNSF-		American Academy of Otorhinolaryngology-Head and Neck Surgery Foundation
Kg	-	Kilogram
Mg	-	milligram
mL	-	millilitre
PBS	-	phosphate-buffered saline
ICs	-	Immune complexes
IgE	-	Immunoglobulin E
IgG	-	Immunoglobulin G
IgM	-	Immunoglobulin M
cGMP -		Cyclic GuanylMonoPhophate
GTP	-	Guanine Tri-Phosphate

DEFINITIONS

ASOM - Otorrhoea within 3 months

CSOM - Persistent otorrhoea greater than 3 months

Purulent OM - Otorrhoea is pus

Mucoid OM - Otorrhoea is mucoid

Congenital hearing loss: hearing loss starting in intrauterine life or prelingual period.

Measles infection: presence of fever, skin rashes with or without other constitutional symptoms.

Mumps: presence of unilateral or bilateral parotid swelling with or without fever.

Meningitis: presence of fever and neck stiffness or clinical diagnosis already made by the physician who managed the pat

ient before referring to our clinic.

Prenatal rubella: history of fever and constitutional symptoms with or without skin rashes in the mother during pregnancy.

Perinatal anoxia: history of delay in crying in a baby delivered in an inadequate health facility or at   home with or without prolonged labour.

Hyperbilirubinaemia: history of yellowness of eyes or skin in neonatal life.

Genetic cause: history of deafness in the family or presence of other congenital anatomic abnormalities, advanced age of mother (more than 35 years) or early onset of hearing loss without any other predisposition.

Ototoxicity: history of consumption of a drug in normal or high doses for at least one week particularly known ototoxic drugs whether prescribed by a physician or quack.

Clinical evidence of malnutrition was determined based on the presence of palor, fluffy hair, body weight less than 70% for age and loss of skin turgidity in the absence of dehydration.

ABSTRACT

Suppurative otitis media or otorrhoea is a major health problem; especially in the first year of life. It could be acute (within 3 months duration) or chronic (greater than 3 months duration). Immunobiologic markers have been identified in otorrhoea, but their role in the outcome of disease remains relatively unknown. This study was aimed at determining the role of these markers in the development of Early Suppurative Otitis Media (ESOM) and the relationship of their levels in the Middle Ear Secretion (MES) and serum to the outcome of Acute Suppurative Otitis Media (ASOM).

One tertiary (University College Hospital, Ibadan) and a community health (Bilal Health Center, Agodi) facilities were purposively selected. Participants included 186 consecutively recruited healthy neonates at birth and 228 children (1 to 12 years) who developed ASOM. The ASOM group was compared with 171 age-matched apparently healthy control subjects from among children at the University College Hospital, Ibadan. Blood samples were collected from the neonates and subsequently monitored monthly for 12 months for development of ESOM. Ten milliliters of Blood (two samples taken at presentation and 3 months after) and MES were collected from the ASOM group who were treated and followed up for 10 months to determine resolution or chronicity (CSOM). The MES was cultured for bacterial infection. Neonatal serum and MES were analyzed for retinol and zinc using high performance liquid chromatography and atomic absorption spectrophotometry respectively. Enzyme Linked Immunoassay was used to determine interferon gamma (IFN-γ) and immunoglobulins (Ig). Data were analyzed using ANOVA at p=0.05.

Incidence of ESOM was 37% among the neonates and the isolates were *Pseudomonas aeruginosa. Staphylococcus aureus* and *Streptococcus spp.* There was no significant

difference in the mean serum IgG between neonates with ESOM and healthy neonates (1180.0mg/ml$\pm$6.3 vs. 1370.2 mg/ml$\pm$9.4). Neonatal cord retinol (0.9µg/L$\pm$ 0.1 vs 1.1µg/L$\pm$0.2), zinc (0.9µg/L$\pm$0.1 vs. 1.1µg/L$\pm$0.2) and IFN-γ (45.3pg/ml$\pm$4.2 vs. l70.2pg/ml$\pm$24.5) were significantly higher in the normal neonates relative to those that developed ESOM. Serum retinol was significantly lower in ASOM children (1.5µg/L$\pm$ 0.6) than healthy control (2.6µg/L$\pm$ 0.3) whereas there were no significant differences in their serum IgG, IgE, IgM, Zinc and IFN-γ levels. In the 46% of ASOM children who developed CSOM, serum levels of IgG was significantly higher (1321.1mg/dL$\pm$21.2) and retinol was significantly lower (1.6µg/L$\pm$ 0.1) than those that had resolved ASOM (666.1mg/dL$\pm$14.4 and 2.1µg/L$\pm$0.1 respectively). The MES levels of IgG (511.5mg/dL$\pm$9.7); IgE (102.0mg/dL$\pm$3.6) and IFN-γ (73.1pg/ml$\pm$12.0) were significantly higher and MES IgA (85.40mg/dL$\pm$13.64) was significantly lower than resolved ASOM (203.4mg/dL$\pm$7.9, 60.4mg/dL$\pm$6.1, 27.2pg/ml$\pm$4.1 and 228.30mg/dL $\pm$16.22 respectively). There were no significant differences in levels of MES IgM, serum IgM, IgA and IgE, IFN-γ and zinc between the two groups.

Reduced levels of neonatal retinol, zinc and IFN-γ were associated with the development of early suppurative otitis media. Reduced middle ear secretion of IgA and IgG; and serum IgG and retinol were determinants of chronicity in acute suppurative otitis media. Immunoglobulins and retinol may be used in monitoring the progression from acute to chronic suppurative otitis media.

Keywords: Suppurative otitis media, Immunoglobulins, Cytokines

Word Count: 493

CHAPTER 1

INTRODUCTION

Otitis media (OM) is highly prevalent worldwide with deleterious effects on intellectual ability, school achievement, speech, and language (Casselbrant *et al.,* 1995). Up to 100% of children in developing communities and 62% of children in more developed communities have been reported to develop OM within the first year of life (Paradise *et al.,* 1997; Bluestone, 2004), and it is expected that the figure would be greater in the developing countries. In Nigeria, a point prevalence of chronic suppurative otitis media (CSOM) of 21.2% had been reported in children in the community (Amusa *et al.,* 2005), while hospital and school prevalence ranged between 1.01% and 4% (Olubanjo, 2008). It accounted for 25% of patient attendance at the otorhinolaryngologic clinic in Nigeria (Lasisi and Ajuwon (2001). In addition, more than 70% of the children with CSOM in Nigeria had developed OM in the first year of life (Lasisi and Ajuwon 2001). In USA, epidemiological studies reported a 90% incidence in children under the age of 2 years (Casselbrant *et al.,* 1995; Paradise *et al.,* 1997) and 60% in preschool attendees (Bluestone 2004). Auinger *et al.,* (2003) reported an 11% increase in the prevalence of early-onset otitis media. They defined early – onset otitis media as development of first episode of otorrhoea at <12 months of age. The lower prevalence figure of OM in Nigeria compared to USA is noteworthy and contrary to expectation. This might be due to many factors: inaccessible healthcare, poverty and local beliefs among others. (Lasisi and Ajuwon, 2001) However, the findings from Nigeria suggested that the development of otitis media in the first year of life – Early otitis media (EOM) may result in the propensity to chronicity and hearing loss (Zakzouk et al., 2002; Lasisi *et al.,* 2007)

There are various criteria of classification and types of OM. The suppurative type of OM is more prevalent in the developing countries while otitis media with effusion

(OME) is predominant in the developed countries (Casselbrant *et al.,* 1995; Paradise *et al.,* 1997; Bluestone, 2004; Amusa *et al.,* 2005). This variation is perhaps a reflection of the disparity in pathogenesis and immunobiological response to the disease; however, the risk factors were identical in the reports. The risk factors identified were low socioeconomic class, bottle-feeding, overcrowding, parental smoking, indoor cooking, daycare attendance, upper respiratory infection among others. (Lasisi et al., 2007) However, it needs be emphasized that reports differed in the effect and significance of these risk factors on the outcome of disease. It was thought this might be related to the type of study and the instruments used in measuring these variables. While the knowledge of these risk factors have been useful in formulating preventive and control strategy, reports of the risk factors are sparse in the developing countries and particularly Nigeria, suggesting a need for this study.

Advances in molecular biology have thrown more light into the pathogenesis of OM and the roles of these risk factors. Recent evidences suggested OM to be primarily inflammatory, the stimuli being bacteria and their breakdown products, viruses and allergy (Auinger *et al.,* 2003; Ryan *et al.,* 2005). In addition, reduced Th1 immune responses at birth have also been proposed to increase the risk for OM and other respiratory infections in the first year of life (Paradise *et al.,* 1997; Lasisi and Ajuwon, 2001; Bluestone; 2004, Amusa *et al.,* 2005). Various bacteria have been cultured by different workers although their contributions to the disease process were still questionable, however, few have reported viral cultures (Vesa *et al*., 2001; Ryan *et al*., 2005). Respiratory syncytial virus, rhinovirus and adenovirus nucleic acids have been identified in effusions (Vesa *et al.,* 2001; Bluestone 2004; Ryan *et al.,* 2005) and evidences of a causal link between OM and viruses are still accumulating. Viral cultures

in middle ear effusion (MEE) have been reported in 24 – 42% of cases (Henderson *et al.,* 1982; Barenkamp, 1986; Vesa *et al.,* 2001; Ryan *et al.,* 2005a).

Allergic reactions in the middle ear have also been postulated to be a cause of OME. The incidence of OME related to allergy was reported to be between 35% to 45% (Henderson *et al.,* 1982; Barenkamp, 1986); and sensitivity to foods and inhalants have been reported in 92.3% and 100% respectively of patients with eustachian tube dysfunction (Ryan *et al.,* 2005b). Although middle ear effusions contained mediators of the allergic response, such as IgE and eosinophil cationic protein, their concentrations in the effusion compared with serum did not suggest that they were produced locally. In addition, controlled studies have not shown increased prevalence of an atopic history or positive skin prick tests in children with otitis media with effusion compared to normal children (Henderson *et al.,* 1982; Barenkamp, 1986; Ryan *et al.,* 2005b).

The generation of cytokines and inflammatory mediators in the nasopharynx and middle ear leading to increased mucosal adherence and colonization by bacteria has also been shown to contribute to the development of AOM. Tumour necrosis factor alpha (TNF α), interleukins (IL-4 and IL-13) have been identified in 77-91%, 67- 97% and 92-100%, respectively, of chronic middle ear effusions (Mogi *et al.,* 1992; Derebery and Berliner, 1997; Barenkamp *et al.,* 2001). These cytokines have been shown to cause increased mucus secretion by up-regulating the mucin genes (Mogi *et al.,* 1992; Willet *et al.,* 1998; Barenkamp *et al.,* 2001) and chemo-attraction of neutrophil (Yellon *et al.,* 1991; Levine *et al.,* 1995; Nassif, 1997). Of clinical significance, their concentrations have been found to correlate with the concentration of endotoxin in the purulent secretion (Yellon *et al.,* 1991; Levine *et al.,* 1995) and viscous effusion (Yellon *et al.,* 1991; Levine *et al.,* 1995; Nassif, 1997; Lin *et al*., 1998; Willet *et al*., 1998; Barenkamp *et al.,* 2001). In addition, high levels of these cytokines in MEE have been correlated

with persistence and chronicity of OM, suggesting the regulation of these cytokines as the possible sites of future therapeutic interventions in OM (Yellon *et al.,* 1991; Levine *et al.*, 1995; Nassif, 1997; Lin *et al.,* 1998; Willet *et al.,* 1998; Barenkamp *et al.,* 2001). Indeed research work in the area of immunobiology of otitis media in the sub – Saharan Africa is still primordial. Therefore the study of the risk factor and immunobiology of otitis media in the sub – Saharan African population is important. It is hoped that this study might reveal the difference in the course of OM between our population and the developed countries and the possible interventions which might be specific to our population.

The objectives of this study are to identify the clinical and epidemiological risk factors associated with early suppurative OM (ESOM), isolating the bacteria, and assessing the serum levels of the prenatal immunobiologic factors such as cytokines, antibodies and nutritional factors (Zinc and retinol); and the role of these markers in the outcome of the disease.

1.1 Rationale for the Study

This study was informed by the persistence of chronic OM in the Otorhinolaryngology out-patient clinic of the University College Hospital, Ibadan, and accounting for more than 25% of the patients. Otitis media is highly prevalent in developing countries including Nigeria with reported prevalence rates 13.8% - 36.2% (Lasisi and Ajuwon, 2001; Bluestone, 2004; Amusa *et al.,* 2005) and accompanying hearing loss in about 50% (Bluestone, 2004; Amusa *et al.,* 2005). The progression to complications and a clear lack of the understanding of the role, perhaps association of some of these risk factors in the patient population and the early onset of disease within one year of life have been other issues suggesting the need for this study. The findings are expected to

facilitate the formulation of preventive strategies and control of hearing loss complicating the disease.

1.2 Hypotheses

1. That higher risk factors are associated with the development of OM within 1 year (early onset) compared to late onset; and early onset otitis media has potential for negative outcome of CSOM using hearing loss and frequency of recurrence of otorrhoea as indices.

2. That infants would be at greater risk of early OM if there had been deranged neonatal serum cytokine, immunoglobulin and malnutrition.

3. That quantitative estimate of middle ear secretion of cytokines and immunoglobulins can be used to determine the course of OM.

1.3 General Objectives

The aim is to establish the possible relationship between quantitative levels of cytokine and immunoglobulin classes in the fetal cord blood; and development of ear infection in the first year of life; and their association with persistence and its chronicity of disease.

1.4 Specific objectives

1. To determine the level of interferon-γ (IFN-γ), immunoglobulin classes (IgG, A and M) and nutritional factors (Zinc and Retinol) in the fetal cord blood and its relationship to the development of acute OM in the first year of life.

2. To evaluate the levels of interferon-γ (IFN-γ) and immunoglobulin classes in the middle ear effusion (MEE) of patients with acute and chronic OM.

3. To determine the relationship between the levels of these biomarkers and development of chronic OM.

4. To establish the proportion of OM relative to elevated middle ear IgE (middle ear allergy).

5. To identify the bacterial isolates in acute SOM

1.5 Research Questions

1. Is the development of acute OM in the first year of life related to the levels of Interferon - γ (IFN-γ), nutritional factors (zinc and retinol) and immunoglobulins (IgG, A and M) in the cord blood samples at birth?

2. Among children who develop acute OM, is progression to chronic OM related to the blood levels of interferon-γ (IFN-γ), nutritional factors (zinc and retinol) and immunoglobulins (Ig G, A and M)?

3. Is there a relationship between middle ear allergy (IgE) and the development and outcome of suppurative OM?

I   II

III   IV
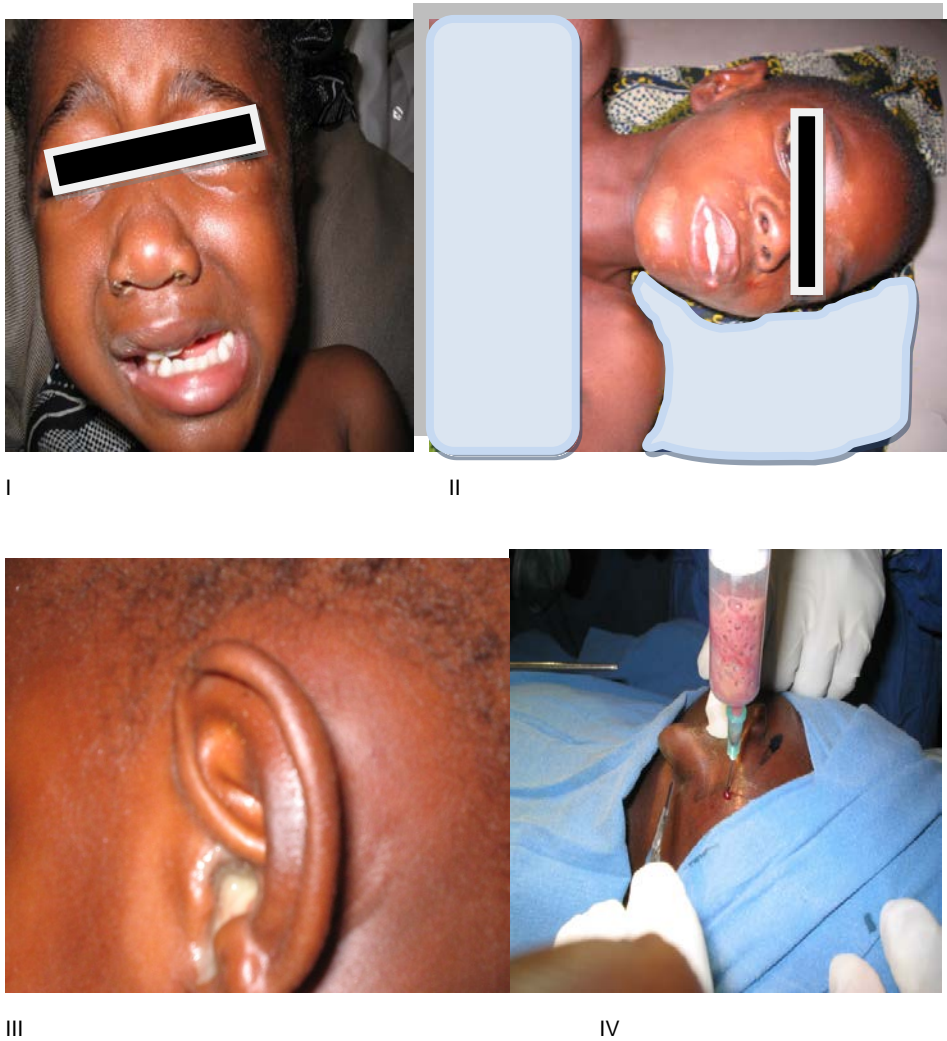
Figure 1.1 Picture showing some of the complications of suppurative otitis media managed in the Department of Otorhinolaryngology, University College Hospital, Ibadan.

i. child with left facial nerve paresis

ii. Intracranial complications – meningitis with complicating SOM

iii. Child with chronic persistent otorrhoea

iv. Mastoid abscess complicating SOM and needle aspiration of purulent materials into the syringe.

CHAPTER 2

2.0   LITERATURE REVIEW

2.1 Preamble

Middle ear inflammation otherwise referred to as otitis media are common among children and form a great bulk of the otorhinolaryngologists' work worldwide (Casselbrant *et al.,* 1995; Paradise *et al.,* 1997; Lasisi and Ajuwon, 2001). The clinical presentation is variable from otitis med

ia with effusion, suppurative, mucoid, purulent and haemorrhagic type (Casselbrant *et al.,* 1995; Paradise *et al.,* 1997; Lasisi and Ajuwon, 2001; Bluestone, 2004; Amusa *et al.,* 2005). This may be a reflection of the pathogenesis and invariably influence the management and outcome.

Up to 100% of children in developing communities and 62% of children in developed communities have been reported to develop otitis media within the first year of life – early otitis media (EOM) (Casselbrant *et al.,* 1995; Bluestone, 2004, Amusa *et al.,* 2005). In the developed countries it ranged between 39% and 84% (Casselbrant et al 1995; Bluestone, 2004; Amusa *et al.,* 2005). Paradise *et al.,* (1997) reported that the proportions developing episode of middle-ear effusion (MEE) between age 61 days and ages 6, 12, and 24 months, respectively, were 47.8%, 78.9%, and 91.1%. This showed that it peaked at about 2 years of age. Auinger *et al.,* (2003) reported an 11% increase in the prevalence of early-onset OM and an 18% increase in the prevalence of repeated OM ($\geq$3 episodes) among children <6 years of age in the United States from 1988–1991 to 1991–1994. This increased prevalence translated to an excess of 561, 000 children with early-onset OM and 720, 000 children with repeated OM in 1991 –1994. The

observed increase in prevalence emphasized the importance of improving our understanding of the epidemiology, diagnosis, and treatment of this common infection.

In an epidemiological survey of 9, 540 children aged up to 12 years in Saudi Arabia, Zakzouk *et al.,* (2002) reported an incidence of Acute Suppurative Otitis Media of 1.05%. The incidence was found to be higher among young children up to 4-years-old and lower in the age group 8–12 years, in addition, a significant male dominance (1.36 and 0.80%), (P<0.01) was observed.

Advances in molecular biologic techniques have proved to be useful adjunct to epidemiology in the understanding of diseases. These have led to innovative approaches in the diagnosis, treatment and vaccine development in OM among other diseases.

In Nigeria, studies reported the prevalence of suppurative otitis media to be between 1.01% and 21.2% (Berman, 1995; Amusa *et al.,* 2005). Besides the work of Amusa *et al.,* (2005) which was community - based, most of these reports were hospital – and school –based. In addition there had not been reports of prospective epidemiologic cohort study of risk factors and molecular biology of suppurative otitis media. Expectedly, the knowledge of these issues will be helpful in planning control and therapeutic measures in suppurative OM.

2.2 Clinical and demographic risk factors associated with suppurative otitis media

Acute otitis media occurs at any age, but primarily affects children between the ages of six months and two years. In the United State of America, 30% of all antibiotics given to children are prescribed for otitis media, and the incidence has been rising over the last 25 to 30 years (Paradise *et al.,* 1997; Bluestone, 2004; Amusa *et al.,* 2005). In addition, it is the most common illness for which children receive medical care in the United States and in most parts of the world. In Nigeria, it accounted for 25% of patient

attendance at the otorhinolaryngologic clinic (Lasisi and Ajuwon, 2001).

Epidemiological studies have reported various predisposing factors. The main classifications being socioeconomic class, childhood infections and environmental factors. According to Homoe *et al.,* (1999), acute OM was found in 2/3 of the children, recurrent acute OM was 20% and COM was 9%. The risk factors identified included parental history of OM (OR = 1.83), sibling (OR = 1.62), and parental plus sibling (OR = 2.56), crowding (OR = 5.55), long period of exclusive breast feeding (> 4 months) (OR = 2.47), and recent acute disease (P = 0.034). Of these, parental history of OM (OR = 1.60; OR = 2.11, respectively) and no recall of breast feeding (p = 0.05) were found to be significant in children with recurrent acute OM and chronic OM.

In addition, chronic OM was found significantly more often in children with two Greenlandic parents (OR = 3.07). Ethnicity in their study reflected socioeconomic class, suggesting that low socioeconomic class was a risk factor for acute OM.

Zakzouk *et al.,* (2002) reported that the prevalence of AOM was higher among children whose parents were cousins compared with non-relative parents (1.38 and 0.74%) (p> 0.01). Poor socio-economic condition also contributed higher rate of acute OM especially those living in the Southern part with inadequate health services. They also found that acute suppurative OM was significantly associated with hearing impairment.

Da Costa *et al.,* (2004) reported that OM cases were more likely to have been exposed to tobacco smoke (OR = 1.51), wood (OR = 1.85) and charcoal (OR = 1.50) household smoke, short term breastfeeding (OR = 1.47), and to living in overcrowded conditions (OR = 1.49). Other works have demonstrated day care attendance by the infant or a sibling, respiratory infection, supine feeding, infectious diseases (respiratory infection, conjunctivitis), and other variables measuring likely exposure to pathogens were important determinants of AOM onset, incidence or duration in the first 6 to 7 months of

life (Stenfors *et al.,* 1992; Lasisi *et al.,* 2007; De Felice *et al.,* 2008; Mandel *et al.,* 2008; Rovers *et al.,* 2008; Vikram *et al.,* 2008). In contrast, there had been other works which found no association between OM and other variables such as breastfeeding, infant daycare attendance, overcrowding and socioeconomic status (Yellon *et al.,* 1991; Nassif *et al.,* 1997; Lin *et al.,* 1998)**,** although overall, the weight of evidence suggested that these socio-epidemiologic risk factors had a role in the predisposition to OM.

2.3 Pathophysiology of Suppurative Otitis Media

OM is inflammatory; the popular theory was that it started primarily following eustachian tube obstruction (ETO) (Mogi *et al.,* 1992; Derebery and Berliner, 1997; Vesa *et al.,* 2001; Ryan *et al.,* 2005b). Eustachian tube obstruction is triggered by an upper respiratory infection (URI) involving the nasopharynx, usually of viral origin. However, allergic and other inflammatory conditions may create a similar outcome. Inflammation in the nasopharynx extends to the medial end of the eustachian tube, creating stasis and inflammation, which, in turn, alters pressure within the middle ear (Sloyer Jr, 1977; Karma, 2002; Masja *et al.,* 2005). Stasis also permits pathogenic bacteria to colonize the normally sterile middle ear space by direct extension from the nasopharynx by reflux, aspiration, or active insufflations. The response is the establishment of an acute inflammatory reaction characterized by typical vasodilatation, exudation, leukocyte invasion, phagocytosis, and local immunological responses within the middle ear cleft to give the clinical pattern of AOM (Ishikawa *et al.,* 1972; Karma, 2002; Masja *et al.,* 2005).

In a minority of otitis-prone children, the eustachian tube is patulous and hypotonic. In children with neuromuscular disorders and abnormalities of the first or second arch, the eustachian tubes are "too open" predisposing to reflux of nasopharyngeal contents into

the middle ear cleft (Sloyer Jr, 1977; Karma, 2002; Masja *et al.,* 2005). Viral infections that attack and damage mucosal linings of respiratory tracts may predispose the nasopharynx, eustachian tube, and middle ear cleft to bacterial infections. This postulate might explain the recovery of viral antigens from middle ear aspirates in children with acute OM, while only rarely is the actual virus isolated. (Barenkamp, 1986; Vesa *et al.,* 2001; Ryan *et al.,* 2005a) Positive viral culture has been reported in 42% - 70% of middle ear secretions (Barenkamp, 1986; Vesa *et al.,* 2001). The viruses identified in effusions were mainly upper respiratory tract viruses such as respiratory syncytial virus, rhinovirus and adenovirus nucleic acids (Barenkamp, 1986; Vesa *et al.,* 2001; Ryan *et al.,* 2005a).

Middle ear mucosal damage by endotoxin secreted by bacterial invaders leading to enhanced adhesion of pathogens to mucosal surfaces has also been implicated (Barenkamp, 1986; Levine, 1995; Ryan *et al.,* 2005b). In addition, allergy and reduced Th1 immune responses at birth have also been implicated in increased risk for OM and other respiratory infections in the first year of life (Barenkamp, 1986; Vesa *et al.,* 2001; Ryan *et al.,* 2005a).

The role of cytokines in the pathogenesis of OM include up-regulating the mucin mRNA expression in the middle ear epithelium and induction of chemo-attraction of macrophages to the site of infections (Henderson, 1982; Barenkamp, 1986; Ryan *et al.,* 2005a). Tumour necrosis factor alpha (TNF α), IL-4 and IL-13 have been identified in 77-91%, 67- 97% and 92-100%, respectively, of chronic middle ear effusions (Henderson, 1982; Barenkamp, 1986; Yellon *et al.,* 1991; Vesa *et al.,* 2001; Ryan *et al.,* 2005a; Ryan *et al.,* 2005b). The concentration of TNF α, IL-1β have been found to correlate with the concentration of endotoxin in the purulent secretion (Henderson, 1982; Ryan *et al.,* 2005b) while IL-8 has been found in higher levels in more viscous

effusion (Nassif, 1997; Lin *et al.,* 1998). In addition, high levels of these cytokines in MEE have been correlated with persistence and chronicity of OM, suggesting the regulation of these cytokines as the possible sites of future therapeutic interventions in OM (Henderson, 1982; Barenkamp, 1986; Vesa *et al.,* 2001; Ryan *et al.,* 2005a).

Allergies have been associated with OME in 35% to 45% of cases (Barenkamp, 1986; Derebery and Berliner, 1997;, Homoe *et al.,* 1999). In addition, sensitivity to foods and inhalants has been reported in 92.3% and 100% respectively of patients with eustachian tube dysfunction (Mandel *et al.,* 2008; Rover *et al.,* 2008). Middle ear effusions contain mediators of the allergic response, such as IgE and eosinophil cationic protein children (Mogi *et al.,* 1992; Derebery and Berliner, 1997, Zakzouk *et al.,* 2002; Vikram *et al.,* 2008). However, from the relative concentrations of the mediators in the effusion and serum it is yet to be confirmed that they are produced locally (Stenfors *et al*., 1992; Zakzouk *et al.,* 2002; Lasisi *et al.,* 2007; Vikram *et al.,* 2008).

2.4 Immunobiologic factors

2.4.1 Immunoglobulins, Nutritional factors and Cytokines In Suppurative OM

The evidence for the development of otitis media supports a complex interaction between infection, allergy and local middle ear immune response to the socio-epidemiologic variables. Reports from previous work have found immunobiologic markers in the middle ear.

Sloyer *et al.,* (1977) reported local production of immunoglobulins in the middle ear. They found specific IgA antibody to measles, rubella, polio and mumps exclusively in middle ear fluid. The mean specific IgA titers were from 8-17 folds higher in middle ear fluid from immunized individuals than in the un-immunized; and this persisted for at least 9 to 19 months after immunization. They concluded that specific immunologic

sensitization of the middle ear mucosa can be achieved by parenteral as well as oral routes of immunization, and specific immunologic memory exists in the middle ear mucosa.

Ishikawa *et al.,* (1972) analyzed 31 middle ear effusions obtained from children with typical serous otitis media for secretory immunoglobulin. All specimens contained IgG and almost all contained IgA and IgM. IgE was found in 2 aspirates and secretory piece in 19. The secretory piece in middle ear effusions appeared to be immunologically identical with that present in other external secretions. The IgG/IgA ratios of paired serum and secretions revealed considerable variations. These studies indicate that the effusion in secretory otitis media was due at least in part to a locally active secretory process with local immunoglobulin production. Hurst *et al.,* (1999) demonstrated increased expression of IL-5 and major basic protein in the middle ear mucosa of patients with otitis media with effusion. They also found extra-cellular protein (ECP) in the middle ear fluid of atopic patients. Recent data by other workers (Mogi and Suzuki, 1997; Tomonaga *et al.,* 1988; Bernstein and Doyle, 1994) illustrated that the middle ear fluid of atopic patients with otitis media with effusion contained more eosinophil, IL-4 and IL-5 and mRNA-positive cells than in non-atopic patients with otitis media with effusion. These findings were suggestive of a role of allergic inflammation in the development of otitis media with effusion.

In an animal model, Bernstein and Doyle, (1994) found that specific mediators of inflammation were released by mucosal mast cells in the nasal mucosa following the interaction of antigen and specific IgE antibody. These mediators increased vascular permeability, mucosal blood flow, and, most important, mucus production. In addition, accessory cell types were recruited by colony-stimulating factors that in turn provided an autocrine-positive feedback for the influx of further inflammatory cells. The

eustachian tube was then effectively obstructed by both intrinsic venous engorgement and extrinsic mucus plugs, isolating the middle ear space from the ambient environment. The net result was the increased exchange of nitrogen into the middle ear mucosa from the middle ear cavity. This led to the development of a significant middle ear under-pressure that disrupted tight junctions; hence transudation of fluids into the middle ear space. The prolonged obstruction of the eustachian tube with mucus would cause middle ear inflammation, mucosal metaplasia, and increased glandular activities, all of which were hallmarks of chronic otitis media with effusion.

Samuel *et al.,* (2008) have reported that IL-1β and TNF-α upregulate mucin secretion from human middle ear effusion in a dose- and time-dependant manner and these effects could be inhibited by cytokine blockade. Kerschner *et al.,* (2004) and Diamond *et al.,* (2000) described mucins as high-molecular-weight glycoproteins with a variety of functions in the middle ear space including protecting the underlying mucosa and assisting with mucociliary clearance. The mucins in middle ear effusion are the components that are responsible for determining the viscosity of the effusion (Samuel *et al.,* 2008; Kerschner *et al.,* 2004; Diamond *et al.,* 2000). In patients with OME mucins are not removed from the middle ear space and contribute to hearing loss and other potential developmental delays. In addition, mucins interact with middle ear pathogens to limit their adherence and invasion of the middle ear mucosa; necessary steps in the development of acute otitis media (Samuel *et al.,* 2008; Kerschner *et al.,.* 2004; Diamond *et al.,* 2000). In addition, different human mucins of various sizes have been identified; most of which are expressed in middle ear effusion (Kerschner *et al*., 2004). However, the process of defining the specific functions of each of these mucin products in the middle ear is still ongoing.

The clinical implication of these functions of cytokines suggests that cytokine inhibitors could mitigate the undesired effects of these inflammatory cytokines, such as increased mucin secretion. Samuel *et al.,* (2008) have demonstrated that interleukin-1 receptor antagonist (IL-1ra) and anti-TNF antibody (TNF ab) significantly inhibited TNF-α and IL-1β stimulated mucin secretion. TNF-α and IL-1β blockade has been used in animal models to reduce shock and mortality in sepsis, reduce the inflammatory response in arthritis, reduce mortality and inflammation in pancreatitis and reduce the inflammatory response in inflammatory bowel disease (Wakabayash, 1991; O'Riordan *et al.,* 1996). Samuel *et al.,* (2008) have also shown in their laboratory, using a chinchilla model, that cytokine inhibition could effect a more rapid and complete resolution from *Haemophilus influenzae* induced otitis media compared with antibiotic treatment alone. More recently, cytokine inhibition has also been used clinically to successfully manage rheumatoid arthritis and inflammatory bowel disease. (First, 2004; Travassos *et al.,* 2005).

The integrity of the middle ear epithelium and secretion of adequate normal mucus are also factors that help in strengthening the middle ear immune system (Bernstein and Doyle, 1994; Zhao and Ross, 1995; Semba *et al.,* 2000). The nutritional factors mainly the vitamins A, C, and E collectively known as the anticancer vitamins and trace minerals like Zinc (Zn) and Selenium (Se) are useful for these functions. Research have shown that these nutrients help in the maturation of the helper T-cells and are valuable in fighting otitis media, colds, flu, among others. (Mogi and Suzuki, 1997; Semba *et al.,* 2000).

Vitamin A plays major role in growth, development, vision and prevention of oxidative tissue damage (Beisel, 1982; Bernstein and Doyle, 1994; Zhao and Ross, 1995; Semba

et al., 2000; Sandau *et al.,* 2002). In investigating therapeutic role of retinol on healing of middle ear mucosa in experimental acute OM, Aladag *et al.,* (2007) found that epithelial integrity was significantly better in the group receiving vitamin A compared to control group ($p < 0.01$). In addition, serum nitric oxide (NO) and malondialdehyde (MDA) levels decreased in the group receiving both antibiotic and vitamin A, while the serum SOD and GSH activity were found to be increased. All of the statistical differences were significant hence they concluded that pretreatment with vitamin A increased antioxidant enzyme activities and reduced the formation of NO and MDA. Manning *et al.,* (1992) studied acute OM in vitamin A deficient pigs after observing an association between vitamin A deficiency and otitis media among children in Micronesia. In the experimental group, they found that 77% of temporal bones showed middle ear pathology. These comprised of subepithelial edema (27%) and frank otitis media (50%) while none of the 15 controls demonstrated middle ear abnormalities.

Similarly, Chole, (1979) found in vitamin A deficient adult and juvenile albino rats, extensive focal squamous metaplasia of the middle ear and eustachian tube mucosa compared with the control group. Keratinization of large areas of normally columnar, ciliated epithelium was observed without frank keratoma formation. In over half of most vitamin A-deficient animals, acute otitis media with effusion was found. They hypothesized that vitamin A deficiency disrupted the mucocilliary clearing mechanism in the middle ear and eustachian tube, which led to effusion and otitis media.

Yilmaz *et al.,* (2004) reported that the blood levels of antioxidants and oxidants before and after the mastoid surgery for otitis media were significantly different when compared with the control group ($P < 0.05$). In the operated group, the blood antioxidant levels increased and oxidant levels decreased significantly after the operation ($P < 0.05$).

However, the levels after the operation never reached those of the control group. Hence they concluded that oxidants and antioxidants played a significant role in the pathogenesis of otitis media with effusion in children. Similarly, Cemek *et al.,* (2005) found statistically significant difference in the serum levels of beta-carotene, retinol, Vitamin E, Vitamin C, and whole blood malondialdehyde (MDA) and GSH levels between the acute OM, acute tonsillitis (AT) and control subjects groups ($P<0.05$). All of the antioxidant vitamins such as beta-carotene, retinol, Vitamin E, and Vitamin C levels were observed to be significantly decreased in the both patient groups than in the healthy control subjects. Experiments in vitro and animal studies suggested that retinoids were important regulators of monocytic differentiation and functions (Hashimoto *et al.,* 1998; Motomura *et al.,* 2001; Cemek *et al.,* 2005). The retinoids were reported to promote cellular differentiation and influence the secretion of key cytokines produced by macrophages, including tumor necrosis factor (TNF-a), IL-1ß, IL-6 and IL-12 (Matikainen *et al.,* 1991; Brook *et al.,* 2005).

Similarly, Zinc is a crucial micronutrient as it influences various aspects of the immune system starting with its effects on the epithelial lining, modulation of the host resistance to several pathogens and various components of innate and acquired immunity (Matikainen *et al.,* 1991; Brook *et al.,* 2005). In addition, severe bacterial illnesses also lead to zinc redistribution (Bondestam *et al.,* 1985; Walker, 2004). There was some evidence that children who experienced recurrent otitis media had lower zinc levels ($p < 0.001$) than healthy controls (Prasad, 1971; Bondestam *et al.,* 1985; Walker, 2004). In addition, Onerci *et al.,* (1997) found that the mean serum level of Zn in the 37 patients with AOM and tonsillitis was significantly lower than in a control group of 28 age and sex matched children. However, whether this alteration in the trace element status caused or fostered recurrent and chronic tonsillitis was not clear. It was thought that if

zinc supplementation was able to help prevent and cure respiratory and diarrheal diseases, it might have a similar protective value against otitis media. However, there are currently no studies relating zinc supplementation to otitis media or hearing loss. This study seeks to find the role of serum zinc in relationship to the development of suppurative OM in Nigerian children.

2.5 Allergy and Otitis Media

In contrast to the other immunoglobulins which are protective, IgE - mediated hypersensitivity promote stasis at the nasopharyngeal end of the eustachian tube and subsequent formation of middle ear effusion (Motomura *et al.,* 2001; Cemek *et al.,* 2005). The allergic aetiology of OM has received attention with numerous reports of high frequencies of allergy in patients with otitis media with effusion and clinical resolution of OM to anti-allergy therapies (Hashimoto *et al.,* 1998; Motomura *et al.,* 2001; Sobol *et al.,* 2002; Yilmaz *et al.,* 2004; Cemek *et al.,* 2005). Alles et al., (2001) reported a 25% and 89% prevalence of allergy respectively in otitis media patients. In addition, they reported positive skin tests to the common inhalant aeroallergens in 57% and an elevated serum IgE in 28% of children with OM. Hurst *et al.,* (1999) concluded that Type I allergy involving a Th-2-type cytokine and cellular profile may be a contributing factor in the persistence of OME in atopic children.

Mogi and Suzuki, (1997), Watanabe *et a*l., (1991) and Chantzi *et al.,* (2006) showed that the pressure - regulating and clearance functions of the eustachian tube were compromised by middle ear histamine. They found that mast cells were distributed in the tubotympanum responding to continuous stimuli to the cavity. In addition, type I allergic reactions of upper respiratory tracts were found to be factors indicative of a chronic state of disease. Sobol *et al.,* (2002) studied inflammatory cells and cytokines in

atopic children with OME and concluded that the predominance of eosinophil, T lymphocytes, and Th-2 mediators in the middle-ear effusions of atopic children provides evidence that allergy might play a role in the pathogenesis of OME. They concluded that the role of type I allergy in OM was potentiation of chronic state of disease rather than a cause.

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┌─────────────────────────────────────────────────────┐
│                      TNF-Alpha                      │
│                          ↓                          │
│              Activate Phospholipase C               │
│                          ↓                          │
│          Hydrolysis of phosphotidylcholine          │
│                          ↓                          │
│            Phosphocholine + Diacyglycerol           │
│                          ↓                          │
│              Activate protein Kinase C              │
│                          ↓                          │
│       Activate and induces Nitric oxide synthetase  │
│                          ↓                          │
│                    Nitric Oxide                     │
│                          ↓                          │
│               Activate Guanyl Cyclase               │
│                          ↓                          │
│                         GTP                         │
│                          ↓                          │
│                        cGMP                         │
│                          ↓                          │
│        Activate cGMP dependant protein Kinase       │
│                          ↓                          │
│    Phosphorylation of serine residues on target     │
│                      proteins                       │
│                          ↓                          │
│              Up regulation of mucin genes            │
└─────────────────────────────────────────────────────┘
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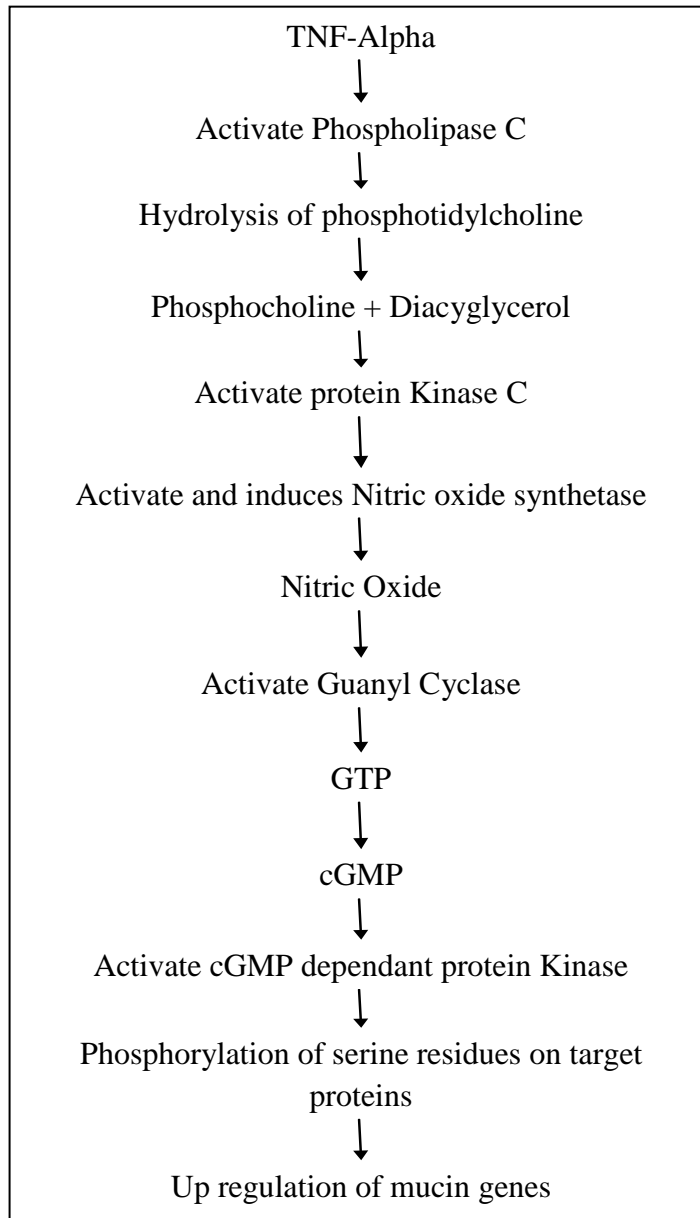
Figure 2.1. Mucin production in OM (Ryanet al., 2005a; Ryan et al., 2005b)

Figure 2.1 explains the cascade of events leading to production of mucus in otitis media. It can be speculated from that immunobiologic markers that up-regulates mucus production like retinol and Th-1 cytokines such as TNF alpha and gamma interferon may enhance development and potentiation of OM.

2.6 Neonatal Immunobiology and Otitis Media

Infancy is a time of rapid immunologic development, and these immunobiologic responses could influence the clinical expression of respiratory viral infections. The immunobiologic state of the infants in neonatal life is a function of the genetic and acquired inheritance from the mother (Bondestam *et al.,* 1985). Maternal transport of immunoglobulins and other biological factors to the foetus mainly occurs after 32 weeks gestation and endogenous synthesis does not begin until several months after birth (Bondestam *et al.,* 1985; Jonsson *et al.,* 2005; Chantzi *et al.,* 2006). Hence cord blood levels of these factors are important in determining the immune response in early neonatal life. Theoretically infectious morbidity and mortality could be reduced by the administration of intravenous immunoglobulin. (Bondestam *et al.,* 1985).

In infancy, IFN-γ played critical role in stimulating macrophages to kill phagocytosed microbes through induction of nitric oxide, and enhanced antigen presentation as well as antiviral effects of CD8 T cells and natural killer cells (Bondestam *et al.,* 1985; Jonsson *et al.,* 2005). This helped in limiting the severity of respiratory illnesses. This hypothesis was supported by case reports of children with deficiencies of IFN-γ production or receptors that experienced more severe clinical manifestations during viral infections (Jonsson *et al.,* 2005). Alternatively, reduced IFN-γ responses might also represent a nonspecific marker of an immature immune response that did not have adequate antiviral activity.

Administration of intravenous immunoglobulin provides IgG that can bind to cell surface receptors, provide opsonic activity, activate complement, promote antibody dependent cytotoxicity, and improve neutrophilic chemo luminescence. (Bondestam *et al.,* 1985; Jonsson *et al.,* 2005; Chantzi *et al.,* 2006)

Jónsson *et al.,* (2005) evaluated in a longitudinal community-based cohort study, the association between maturation of immunoglobulins and mannan-binding lectin (MBL) responses and disease manifestations in the first 4 years of life. They found that sustained low levels of IgA proved the strongest single indicator of susceptibility to recurrent otitis media and respiratory tract infections; and this condition was also associated with low production of IgG subclasses. Low levels of IgA within the normal range might reveal disease susceptibility not detected by conventional criteria. Slow maturation of immunoglobulin appeared to be the main factor of susceptibility during childhood, but a strong corollary role for MBL is indicated by the high levels produced during childhood and the precipitation of disease in children with low levels of MBL and immunoglobulin.

Ishizaka *et al., (*1994) measured the serum immunoglobulin levels and naturally occurring antibody titers against *Streptococcus pneumoniae* in seven children aged 1– 1.9 years with recurrent pneumococcal acute otitis media. The mean antibody levels of anti-pneumococcal IgG_1 and anti-pneumococcal IgG_2 were significantly lower in patients when compared to those of healthy controls and children who had less frequent episodes of acute otitis media. Following treatment with intravenous immunoglobulin for 6 months, anti-pneumococcal IgG_1 and IgG_2 antibody levels increased and the number of episodes of AOM decreased in all patients. These results suggested that delayed maturation of anti-pneumococcal antibody production caused recurrent acute otitis media and this condition was corrected by intravenous immunoglobulin therapy.

Soltan and Jenkins, (1983) found that cord blood zinc concentrations in congenitally abnormal babies were lower than in the control babies. Hence they concluded that low plasma zinc may be an associated factor in the aetiology of fetal abnormality.

Nasrat *et al.,* (1992) reported low levels of plasma zinc concentration in 10 fetuses with symmetrical growth retardation, but the levels were not significantly different from that in the normal control foetuses. However, they concluded that in fetuses with symmetrical intrauterine growth retardation, low plasma zinc was probably a parallel phenomenon and not necessarily an aetiological factor. Zinc deficiency during foetal development was documented to cause intra-uterine growth retardation and also impaired postnatal immune functions making these babies more susceptible to severe infections (Bondestam *et al.,* 1985; Watanabe, 1991; Mogi and Suzuki, 1997; Chantzi, 2006).

Rahman *et al.,* (1996) examined the effect of vitamin A supplementation on cell-mediated immunity among infants younger than 6 months in Bangladesh. Their results showed cell-mediated immunity responses were improved among infants with adequate serum retinol concentrations (>0.7 µmol/L) after supplementation, but there was no improvement among children with low serum retinol levels after supplementation. Similarly, results from the trial in Indonesia showed a significant reduction in mortality rates only in infants with normal birth weight and who were not malnourished. (West, 1995). Vitamin A deficiency decreased the amount of secretory antibody in mucosal secretions (Beisel, 1982; Biesalski *et al.,* 1996). There was evidence indicating that sIgA to pneumococcal capsular polysaccharide, which interferes with the adherence of pneumococci to mucosal epithelial cells, appears in the secretions of infants as early as 6 months of age. (Soveri, 1999).

In summary, the overall findings in risk factors and role of immuno-biologic factors in development of OM suggest the need for further research into the role of these biomarkers in the course of suppurative otitis media.

CHAPTER 3

METHODOLOGY

3.1 Study design

This was a prospective study of the socio-epidemiological risk factors and the immunobiology of early otitis media. The design was in 2 parts: The first involved a prospective questionnaire study of the risk factors associated with early otitis media. The second was a longitudinal cohort study of the immunobiological markers in the fetal cord blood associated with development of OM in first year of life and the role of these markers in the outcome of OM.

3.2 Study sites

The study took place in the University College Hospital, Ibadan and Bilal Medical Mission, Agodi, Ibadan, in Oyo State, Nigeria. The obstetrics facilities of the University College Hospital are specialized centres with an estimated average delivery of 100 per month. While Bilal Mission is a primary care center managed by a retired trained nurse and supervised by a general practitioner. The service is mainly maternity with an estimated average of 60 deliveries per month. The patient distribution in the University College Hospital is mainly high social class compared to Bilal Mission which is mainly low social class. The University College Hospital Ibadan is located on latitude 7.4069N and longitude 3.9024E; while Bilal Mission, Agodi, Ibadan is lcated on latitude 7.4064N and longitude 3.8992E.

3.3 Study duration

24 months from March 2007 to February 2009.

3.4 Sample size

This was done using an online module 73

Figure 3.1. An online module showing the estimation of the sample size for the recruitment of subjects for Acute Suppurative otitis media.

http://biostat.hitchcock.org/MeasurementError/Analytics/SampleSizeCalculationforLogi

sticRegression.asp

Computerized model for calculating sample size of cohort study by using varying standard deviation and assumptions for other elements in the formular shown under the title.

The upper curve is for questionnaire studies with high margin of error while the lower curve is for laboratory studies with lower margin of error, so this study used the lower curve. The estimated sample size was 245 mothers/neonates; however, 5%(22) subjects were added making 267) subjects were recruited to compensate for loss to follow up.

3.5 Participants recruitment

The participants' recruitment took place in 4 phases according to the objectives of the study with one phase linking to the other. The first phase was the study of the epidemiologic risk factors associated with development of suppurative otitis media in children. This was embarked upon in order to provide guidance on the likely immunobiologic markers to be investigated. This was followed by recruitment of pregnant mothers in the third trimester of pregnancy, withdrawal of fetal blood and follow –up of the neonates for one year for the detection of early suppurative otitis media. Finally, children with acute suppurative otitis media were followed up to determine those subjects who had persistence of disease for more than 3 months (Chronic suppurative otitis media) and the serum markers were determined. The recruitment was as follows:

3.5.1 Study of the epidemiologic risk factors of early onset otitis media
A survey of the risk factors in children with chronic recurrent suppurative otitis media presenting to the hospital was carried out. For the purpose of this study, the definition of early onset otitis media was based on the history of ear discharge within the first year of life and CSOM as chronic perforation with inflammation of the middle ear persisting for

more than 3 months (Lasisi *et al.,* 2007). The study took place in 2 hospitals in Ibadan: University College Hospital and Bilal Medical Mission. All these health facilities were located in sub-urban areas of the country. The study started following ethical approval (see Appendix 1) by the Institution Review Board of the University of Ibadan/University College Hospital, Ibadan; consent was taken from the parents and the questionnaires were administered to the parents orally. The children were examined for presence of the disease and the risk factors. The information sought in the questionnaire included biodata, age at first episode of ear suppuration, number of episodes in 18 months, the number of people in the household, exposure to indoor cooking, allergy and socioeconomic status of the parents. The examination of the children emphasized on the nutritional status, presence of upper respiratory tract infection, malnutrition, adenoid and allergy.

All the subjects had nasal smear cytology for eosinophillia while plain radiograph of the postnasal space was done in the subjects with snoring to confirm adenoid hypertrophy.

Conversation and tuning fork methods were used to assess hearing. For those with poor response to tuning fork, pure tone audiometry was done using a computer audiometer (BA 20 Kamplex) in a sound-proof (acoustic) booth in the otorhinolaryngology outpatient clinics (calibration ISO/DP 389–1983). Hearing was tested at the frequencies 250–8000 Hz for each ear separately.

The controls were 100; made up of 15 children of hospital workers, 20 children of parents visiting the hospitals and 65 from school children, all of whom volunteered that there had been no episodes of otitis media (OM) in the past and audiometry was done.

The socioeconomic class was defined as high (I and II), middle (III) and low (IV and V) based on occupation, income earning and education of the parents (Office of population censuses and survey, (1970); Lasisi *et al*., 2007).

3.5.2 The role of neonatal immunobiology in the development of early suppurative otitis media

For this study, consecutive eligible and consenting pregnant mothers in the last trimester of pregnancy were counseled and recruited over a 6 month period. The participants were normal pregnant women who had antenatal care, labour and normal delivery at the University College Hospital and the Bilal Mission Hospital Ibadan. The inclusion criterion was normal pregnant mothers as adjudged by the obstetrician while the exclusion criteria included pregnant mothers with hypertension, diabetes, malnutrition, systemic disease, autoimmune, infectious, or inflammatory diseases.

Using oral interview and confirmation in the hospital record chart, the detail of the tetanus vaccination, fever, and antenatal care in pregnancy were documented. The gestational age was calculated with the Naegele's rule, using the lower limit. The mothers were followed up to the labour and delivery. At delivery, the biodata of the neonate were noted such as Apgar score, weight and the cord blood was taken within 24 hours of delivery (proforma, see Appendix 2 and 3). This study had ethical approval from the University of Ibadan Ethical committee (see Appendix 4)

3.5.3 Study of the role of immunobiologic markers on the outcome of otitis media

The neonates who developed otitis media above were also added to other children with otitis media recruited. These children were recruited from the General Out-patient department and the Otorhinolaryngology outpatient Clinic of the University College Hospital, and the Bilal Medical Mission, Ibadan. The inclusion criteria were children under the age of 12 years with acute mucoid and purulent otitis media using the reference of 3 months as cut off for acute OM (Bluestone *et al* 2002, Lasisi *et al* 2007). Informed consent was obtained from the parents and recruitment commenced. The subjects had history taken, and examination of the ear, nose and throat with a hand held

otoscope and Shirom lamp and head mirror. They were differentiated into POM if the otorrhoea is pus and MOM if it is mucoid. After diagnosis, the patients were offered suction ear toileting, topical wick dressing, and nasal decongestants for 2 – 4 weeks. Oral antibiotics for 1 week were added to the treatment if otorrhoea persisted after 4 weeks of topical dressing. All the patients were followed up for 12 months. The criteria for resolution of OM and commencement of healing of tympanic membrane were taken as the cessation of otorrhoea and otoscopic finding of neo – membrane formation closing up the tympanic membrane perforation.

The control subjects, who were comparable to subjects in age and sex, were randomly selected using convenient sampling from among healthy children visiting the hospitals, children of hospital workers and school children. Blood was taken for immunoglobulin, retinol and zinc estimation.

3.5.4. Study of the role of Allergy in the development of suppurative otitis media

The profound significance of allergy as an epidemiologic risk for chronic suppurative otitis media informed the need for further study role of specific allergy and serum IgE in the outcome of suppurative otitis media. In doing this, history of allergy was enquired by asking questions on hypersensitivity to house dust, feather, grass, pollens and smoke from fire.

Each of the patients with history of allergy had nasal smear cytology for eosinophil to further confirm nasal allergy and skin sensitivity test. The control subjects comprised of children who never had a history of ear discharge. These control subjects were selected among children from parents (on visit to the hospital), children of hospital workers and school children.

3.6 Collection and Storage of Cord Blood Sample

Within the 24 hours of delivery, the fetal weight and gestational age were recorded and cord blood samples of the neonates were taken from the umbilical vein using a 21G needle and 5 ml syringe, and introduced into a non – heparinized bottle. This was centrifuged at 1500 x g for 10 minutes. After clot extraction, the serum was separated and stored at -80∘C.

3.7 Follow up of Neonates

These neonates were followed up monthly for at least 12 months. They were seen in the outpatient clinic and the mobile telephone was also used to monitor some of them in order to reduce the chance of missed cases. All these infants received vitamins, minerals supplementation during the follow up period.

Cases were defined as those who developed otorrhoea (ESOM) while the controls subjects were those without otorrhoea within the study period. Middle Ear Secretion

(MES) were aspirated from the cases with ESOM and stored at -80°C. In addition, swab was also taken for the bacterial isolates, this was transported to the laboratory immediately.

The subjects with otitis media were treated with systemic and topical antibiotics; nasal decongestants and ear dressing. They were followed up every week to determine the healing of OM which was defined in this study as the cessation of otorrhoea.

After treatment, the AOM groups were re-evaluated 3 months after onset of secretion (duration cut-off for COM). Those with persistent secretion had a repeat pippeting of MES for determination of the cytokine and immunoglobulins levels. The two groups were followed up every month for 1 year.

Follow-up involved brief history regarding URI, allergy and OM and full physical examination of the ear, nose and throat and the nutritional status of the subjects was assessed.

This part of the study started on the February 2007 and ended on the December, 2008.

3.8 Blood and Middle Ear Secretion (MES) collection procedure

The blood samples of the subjects with otitis media were collected through a venepuncture in a medium vein in the arm (brachial vein) using a 21/22G needle after sterile swabbing (The use of a 21/22G needle will reduce pain to barest minimum).

The MES were pipette into a sterile centrifuge bottle by a non-touch technique. Immediately after collection, the two samples (MES and blood) were transported in wet ice at -10∘C from the Clinic and serum was extracted from the blood by centrifuging at 1500xg for 10minutes and the use of a Pasteur pipette. The samples were then stored at -80ºC. The samples were used for quantitative analysis of cytokines and immunoglobulin classes.

Enzyme-linked assay techniques were employed for the quantitative estimation of the interferon-γ (IFN-γ), IgE in the MES and serum of subjects. Normal controls were selected among (i) those without otitis media for serum quantitative estimation of the same factors.

The serum levels of these cytokines, immunoglobulins and nutritional factors (retinol and zinc) were compared between controls and subjects.

3.9 Culture of Bacterial isolate

This was done according to standard techniques as reported by Ako-Nai *et al.,* (2002) and Oni *et al.,* (2002). The swab of the MES were inoculated onto a sheep blood agar and incubated aerobically at 37ºC for 18-24 hours. Gram staining was done using

standard Gram staining technique. After 18-24 hours, visual examination was done for bacterial growth. In cases where there were growths, discrete colonies were selected for morphology and then biochemical tests were carried out to identify the different species and the sensitivity testing of these samples was alsocarried out.

3.10 Quantitative Measurements of Cytokine and Immunoglobulin Classes

The quantitative estimate of the cytokines and specific immunoglobulins were determined using the ELISA technique as described by the manufacturer while total immunoglobulin classes were determined using the single diffusion

3.11 Quantitation of Immunoglobulins Classes

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

plotted on a semi-log graph paper and the concentrations of the test and control samples were read off the standard curve.

3.12. Quantitation of Immunoglobulin E

The total Immunoglobulin E was quantified by the enzyme linked immunosorbent assay method (Hurst *et al.,* 1999b; Sobol *et al.,* 2002). This was done with a mixture of 3% noble agar prepared in phosphate buffered saline (PBS, pH 7.2) and 1 milliliter of each antiserum (anti-human immunoglobulin class). Several dilutions (25%, 50%, and 100%) of the standard serum were prepared in PBS. Using a 5ml micro-dispenser the sera, middle ear effusions and standards were applied to the punched wells and the plate for Ig E estimation was put into a humid chamber and incubated for 18 hours. The assays were validated and diameters of the precipitation rings were measured to the nearest 0.1mm using eye precision viewer. Standard curve for the IgE was plotted on a semi-log graph paper and the concentrations of the test and control samples were read off the standard curve.

3.13 Skin Sensitivity Test

Skin sensitivity prick test was carried out among the following allergens: house dust, house dust mite, mould, cockroach and poultry feather. The forearm of the subjects were marked out for allergen application, then sterilized with spirit swab and allowed to dry. Positive and negative controls allergens at room temperature were pierced into the epidermis using a special lancet. The diameters of the wheal and flare reaction were measured with a meter rule after 20 minutes. The response was defined as positive when the diameter was more than 3 millimeter.

The diameters were recorded for each allergen and were compared with the controls to grade the severity of the response.

3.14 Quantitation of Serum retinol

Sample extraction and determination for retinol was done by measuring 0.125μL of serum and diluting up to 500μL volume with ultra pure water. An antioxidant (10g/dL Ascorbic acid) was added. The mixture was shaken for 15 minutes, followed by 5 minutes of sonication. Triton was added as detergent and an internal standard (400μL of acetonitrile) was added and mixed properly. To this mixture, was added 400μL of n-hexane, the moisture was shaken for 5 minutes and centrifuged for 2 minutes at 8000 revolution per minute. The supernatant was collected and retinol determination was done using the high performance liquid chromatography (HPLC) method (Craft *et al.,* 2000).

3.15 Assay of Interferon Gamma Assay (IFN-γ)

The wash buffer, substrate solution, IFN-γ standard and the calibrator diluents were reconstituted as specified. Into each well, 100μL of assay diluents was added; this was followed by addition of 100μL of the standard, sample and control and then incubated for 2 hours at room temperature. The aspirate was washed 4 times and 200 μL of conjugate was added to each well and incubated for 2 hours at room temperature. The aspirate was then washed 4 times and 200μL of substrate solution was added, incubated at room temperature for 30 minutes protected from light. The Enzyme linked immunosorbent assay method was employed to assay IFN - γ using the Thermolabsystems Multiskan® ex machine. The colour change was read at 450nm immediately. The standard curves for the various classes of immunoglobulins were plotted on a semi-log graph paper and the concentrations of the test and control samples were read off the standard curve.

3.16 Determination of Plasma Zinc

Serum was deproteinised using 5 ml of 10% trichloroacetic acid in 0.1% lanthanum solution. Zinc levels were determined in the resultant supernatant using flame atomic absorption spectrophotometry (Model 205 Buck Scientific, East Norwalk CT, USA 06855). All reagents and materials used for the analysis were free of zinc contamination and the plastics used for the analysis were previously washed with Hydrochloric acid.

3.17. Quality control and regular calibration of instrument

The control and standard sera were included in the analysis at every sera assay to ensure reliability and quality of the procedure. An initial pilot study was conducted to test all instruments, this was then followed by a preliminary statistical analysis to detect outliers and correct factors.

3.18. Definitions

1-Year Outcome Variables - Acute otitis media was defined as a parental report of otorrhoea and fever and examination finding of ear discharge and perforation of the tympanic membrane in the first year of life.

Acute suppurative otitis media- Presence of ear discharge of duration up to/less than 3 months.

Chronic suppurative otitis media - Presence of ear discharge/perforation of tympanic membrane of duration more than 3 months.

Allergic reaction - Presence of history of persistent sneezing on exposure to irritants with prominent nasal eosiniphillia with or without elevated serum Immunoglobulin E.

The socioeconomic class was defined as high (I and II), middle (III) and low

(IV and V) based on occupation, income earning and education of the parents (Office of

population censuses and survey, (1970); Lasisi *et al*., (2007).

Upper respiratory tract infection - Presence of cough or rhinorhoea or nasal stuffiness or sorethroat with or without fever.

Malnutrition- Clinical evidence such as fluffy hair, palor, small weight for age.

Viral infection – Patients with positive antibody to the viruses tested.

3.19. Statistical Analyses

(i).In the study of the epidemiologic risk of otitis media, the main outcome variable was early onset otitis media which was coded as 1 and otitis media after 1 year of life coded 0 for the purpose of logistic regression analysis. Univariate analysis consisted of cross-tabulation with generation of odds ratio (OR) and its 95% confidence interval. The number of subjects with early onset otitis media was analyzed out of the total subjects and the risk factors, frequency of recurrence and hearing loss were analyzed between the 2 groups.

The prevalence, type and severity of hearing loss, association with age at onset of OM, frequency of recurrence, socio-economic status and recurrent upper respiratory infections were determined.

The number of people in the household was divided into 2 groups using the median value of all the people in all households of the participants as the cut – off value. The variables were further subjected to multivariate analysis by logistic regression. Stata software was utilized for all statistical analysis. Hypothesis testing for level of statistical significance was done using 95% confidence interval and P value < 0.05.

ii. In analyzing the role of neonatal immunobiologic markers on the development of otitis media in the first year of life, the main outcome variable was the development of otitis media within the first year of life while the dependent variables were the serum

levels of zinc, retinol, IgG and IFN γ in the neonates. The data was initially explored using the stata software, variables were analyzed by unpaired t-test both for equal and unequal variance using the variance ratio function of the Stata software to determine the appropriate use of the Satterthwaite's correction for the degrees of freedom. The logistic regression analysis was used to control for maternal and neonatal confounding factors. The stata software$^{®}$ was used and the level of statistical significance was set at p< 0.05 for all the analyses.

iii.In assessing the immunobiologic markers and otitis media, the main outcome variables were the presence of ear discharge in the children while the explanatory variables were fetal weight, gestational age, family history of ear discharge, quantitative levels of immunoglobulin E, G and M and cytokines, IFN γ; in the fetal blood at birth; and in the middle ear effusion and sera of subjects and the hearing function.

iv. The main outcome variables for the assessment of allergy were the skin sensitivity to allergens and the IgE levels in serum and middle ear secretion. The variables were analyzed by unpaired t-test both for equal and unequal variance using the variance ratio function of the Stata software to determine the appropriate use of the Satterthwaite's correction for the degrees of freedom. Level of statistical significance was at p< 0.05 for all the analyses.

v. Assessing the role of micronutrient

The main outcome variables were the serum levels of retinol and Zinc in patients with AOM and COM. The data was initially explored using the Stata software, variables were analyzed by unpaired t-test both for equal and unequal variance using the variance ratio function of the Stata software to determine the appropriate use of the

Satterthwaite's correction for the degrees of freedom. Level of statistical significance was at $p < 0.05$ for all the analyses.

3.19.1. Univariate Analysis

All the variables measured were cross tabulated against the main outcome variables and also with the main exposure variables to detect possible confounders which were adjusted for in the multivariate analysis.

3.19.2 Outcome variable: Ear Discharge

Explanatory Variables were: fetal weight, gestational age, family history of ear discharge, quantitative levels of immunoglobulin E, G and M; and cytokines IFN; in the fetal blood at birth; and in the middle ear effusion and sera of subjects and the hearing function.

The crude measures of association such as odds ratio or hazard ratio were obtained.

3.19.3 Multivariate Analysis

The Cox Hazard regression model and the logistic regression model were utilized in testing associations in this study. In the multivariate model building, adjustments were made for variables such as fetal weight, gestational age, breastfeeding in the first year of life (yes versus no), maternal age, and maternal/paternal history of otitis media, fetal serum cytokines and immunoglobulins. The odds ratio and Hazard ratio was utilized as the main measures of association. For all these analyses, the 95% confidence interval of all estimates and the p-values were calculated.

3.20. Ethical Considerations

Ethical clearance was sought and given from the University of Ibadan/University College College Hospital Ibadan, where theresearch was carried out. Each participant

was seen privately in the consulting room and the purpose and the extent of the study explained in details, verbally and very carefully both in English and the local Language to the individual participant. Freely given informed consent was obtained from them before enrolment in the study. Only participants who agreed voluntarily to participate were recruited into the study and the true identity of the participants was kept secret.

The transportation of the patients to the hospital for monthly follow-up and financial cost of estimation of the cytokines was paid by the research project. Pain was reduced to the barest minimum by using a size 21/22G needle for venepuncture.

Complications arising from standard treatment were treated and such patients were excluded from the study.

Refusal to participate did not in any way jeopardize patient from standard consultation and treatment.

3.21. Informed Consent form

My name is _____, of the Department of Medical Microbiology and Parasitology, College of Medicine, University of Ibadan.

Information on the study: You will be asked about the history of upper respiratory infection, cough and ear infection in your child/ward followed by general examination and examination of the ear, nose and throat. Specimen sample of ear and nasal discharge and 5ml of blood will be collected. Pain will be reduced to barest minimum by using a size 21/22G needle for venepuncture and the procedure will be discontinued if any adverse reaction is noticed from the skin allergy prick test.

The information will be treated confidentially and the result will be used for treatment and research purpose. You are free to refuse to participate in this programme and your refusal to participate will not in any way jeopardize your consultation and treatment.

The benefit to you will include free test and free antibiotic treatment.

You will be required to show your willingness to participate in this study by appending your signature below.

Consent: Now that the study has been well explained to me and I fully understand the content of the study process, I will be willing to take part in the programme.

Signature/Thumbprint of participant _____

Signature/Thumbprint of subject (Parent) _____

Signature/Thumbprint of witness   _____

CHAPTER 4

4.0   RESULT

4. 1. Epidemiologic risk factors of early onset otitis media

The survey studied 189 children with chronic suppurative otitis media (CSOM), comprising of 123 males and 66 females (1.5:1). The age ranged between 6month to 150 months, mean of 59.2 (SD = 44.6).

Of the 189 subjects with CSOM, 136/189 (70.0%) had the first episode of otitis media before 1 year of age (early onset OM). The frequency of recurrence of otorrhoea was between 1 and 9 in 18 months, with a median frequency of 3.

The early onset group had greater frequencies of recurrences than the later onset group (figure 4.1.1), however, statistical analysis did not find correlation between the frequency of attack of otorrhoea and age at onset of otitis media (OR = 1.03, 95% CI = 0.88 – 1.19, P = 0.75).

Sociodemographic survey revealed that early onset Otitis media was found in 110 of Low social class, and later onset in 40, figure 4.1.2. Allergy was seen in 74 subjects, which was found in 54 of the early onset OM and 20 of the later onset. Adenoiditis/adenoid hypertrophy was seen as risk factors in 66 of the early onset and 17 later onsets OM. The number of people in the household was found to be above the median in 125 of the early onset and 11 of the later onset.

Bivariate analysis revealed significant correlation between early onset of OM and hearing loss (OR = 0.28, 95%CI = 0.133 – 0.57, p = 0.01), but there was no correlation with increased frequency of otorrhoea (OR = 1.03, 95%CI = 0.88 – 1.19, p =0.75). The significant risk factors are number of people in household (OR = 4.13, 95%CI = 1.81 – 9.39, P = 0.01), allergy (p = 0.03) and low social status (p = 0.01) (tables 4.1.1 and 4.1.2). Bottle-feeding, adenoiditis/adenoid hypertrophy, indoor cooking and upper

respiratory infection was not found to orrelate with early onset OM.

Hearing loss was confirmed by audiometry in 89/189 (47%) subjects and was conductive in 73 (82%) and sensorineural (SHL) in 16 (18%). The duration of CSOM ranged from 4 weeks to 12 years (mean $\pm$ SD = 4 yrs $\pm$ 2.04). However, among those with SHL, the range was 5–12 years (mean $\pm$ SD = 9 yrs $\pm$ 6.21). Hearing loss was mild in 37%, moderate/moderately severe in 10% and in none was it severe or profound (Table 4.1.3).

Regarding socio-economic status, 61/89 (69%) of subjects were of low social class, 13/37 (35%) middle class and 15/63 (24%) high social class, compared with controls made of 55 (55%) high, 20 (20%) middle and 25 (25%) low social class (Fig 4. 1. 2).

Of the 89 patients with hearing loss, 72 (80.90%) had developed OM within the 1st year of life. Hearing loss was detected in 68/133 (51%) of those who developed CSOM within the 1st 6 months of life and in 4/22 (18%) who developed it after 6 months of age (Fig 4.1. 3).

The frequency of OM was 0–3 in 54 (29%), 4–6 in 21 (11%) and 7–9 in 14 (7%) (Table 4.1.4). Hearing loss was seen in 54/126 (43%) of those who had had 0–3 attacks in the past 18 months, 21/40 (53%) who had had 4–6 attacks and 14/16 (88%) who had had 7–9 attacks.

The treatment offered included aural suction toileting followed by daily topical dressing with an antiseptic (flavine in spirit) gauze wick and a topical nasal vasoconstrictor. Systemic and topical antibiotic ear dressings were added when there was no improvement in the otorrhoea after 2–3 weeks. Cessation of otorrhoea was achieved in 117 (62%) on topical treatment while the remaining received added systemic/topical antibiotic therapy and cessation of otorrhoea was achieved in all the remaining between 4 and 10 weeks.

There was a significant correlation between hearing loss and socio-economic status (r=0.14, p=0.02) and age of onset (r=-0.04, p=0.02), but no correlation with upper respiratory infection (r=0.05, p=0.36), and frequency of attack (r=0.07, p=0.35) (Table 4.1.5).

Table 4.1.1. Multivariate analysis comparing subjects with early onset otitis Media (<1 year) and late onset group

Variables	OR (Odds Ratio)	95%Confidence Interval	p value
Bottle-feeding	0.96	0.45 – 2.04	0.92
Adenoiditis/Adenoid Hypertrophy	0.57	0.258 – 1.28	0.18
Malnutrition	0.62	0.29 – 1.35	0.23
Allergy	0.51	0.23 – 1.10	0.09
Low Social status	0.70	0.31 – 1.55	0.38
Number of People in household (> 10 vs. < 10)	4.13	1.81 – 9.39	0.01
Indoor Cooking	2.34	1.18- 4.66	0.01
Frequency of attack	1.03	0.88 – 1.19	0.75
Hearing Loss	0.28	0.13 – 0.57	0.01

Table 4.1.2: Univariate analysis comparing the risk factors between early onset otitis Media with later onset group

Variable	Later onset OM n (%)	Early onset OM n (%)	p value
Adenoiditis/Adenoid Hypertrophy	17(20.48)	66(79.52)	0.60
Allergy	20(27.03)	54(72.97)	0.03
Low Social Group	40(26.67%)	110(73.33)	0.01
Number of people in Household > 10vs $\leq$ 10	11(8.09)	125(91.91)	0.01
Upper Respiratory Tract infection	40(27.03)	85(62.5)	0.42
Hearing loss	17(17%)	83(83%)	0.01
Bottle feeding	22(24.4)	68(75.56)	0.09

Age in months
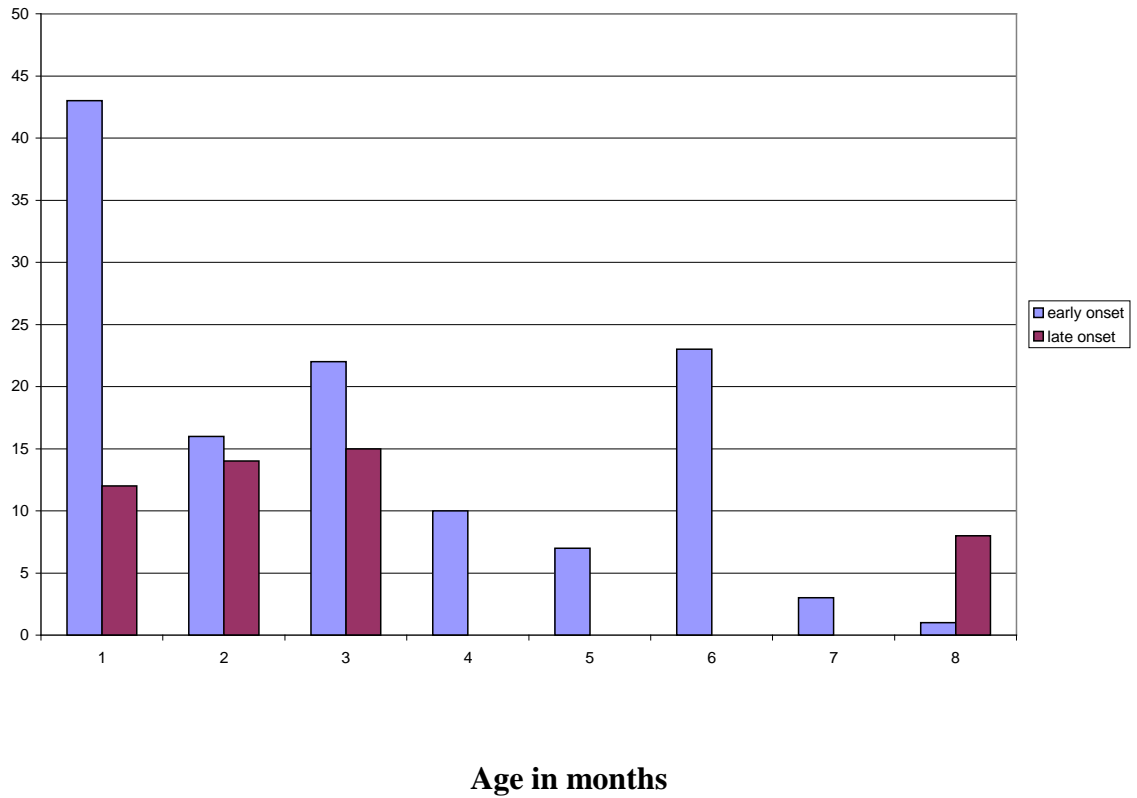
Figure 4.1.1. Bar chart showing the frequencies of otorrhoea between subjects with early onset and later onset otitis media.
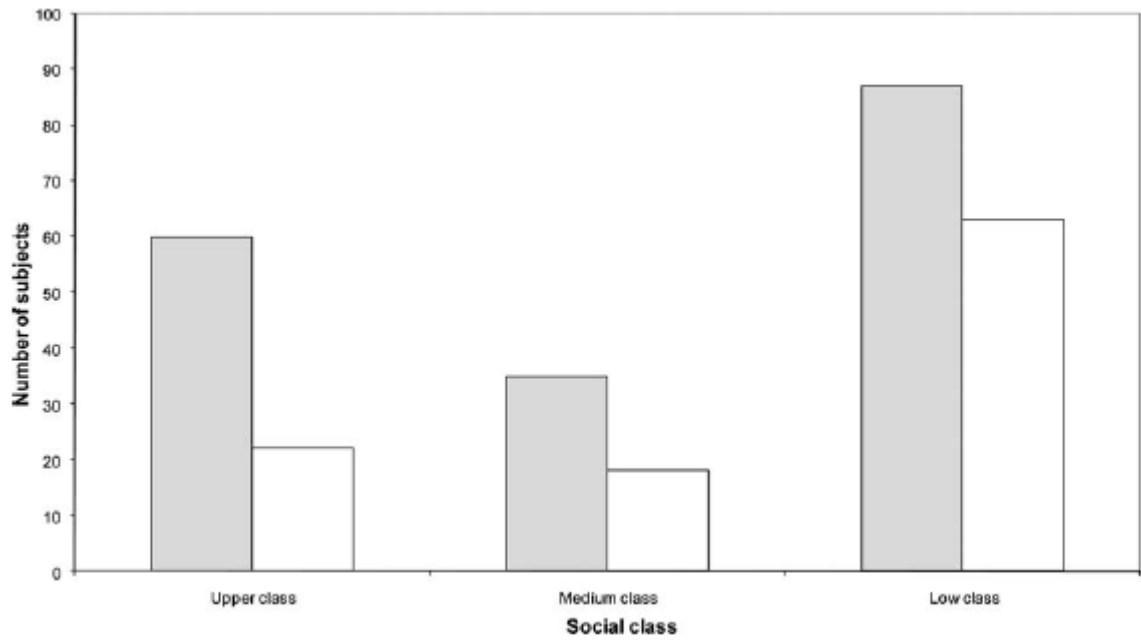
FIG. 1. Hearing loss and social class (■ no, □ yes).

Figure 4.1.2: Hearing loss and social class distribution among subjects with ASOM

Figure showing the Age at onset and presence of Hearing Loss
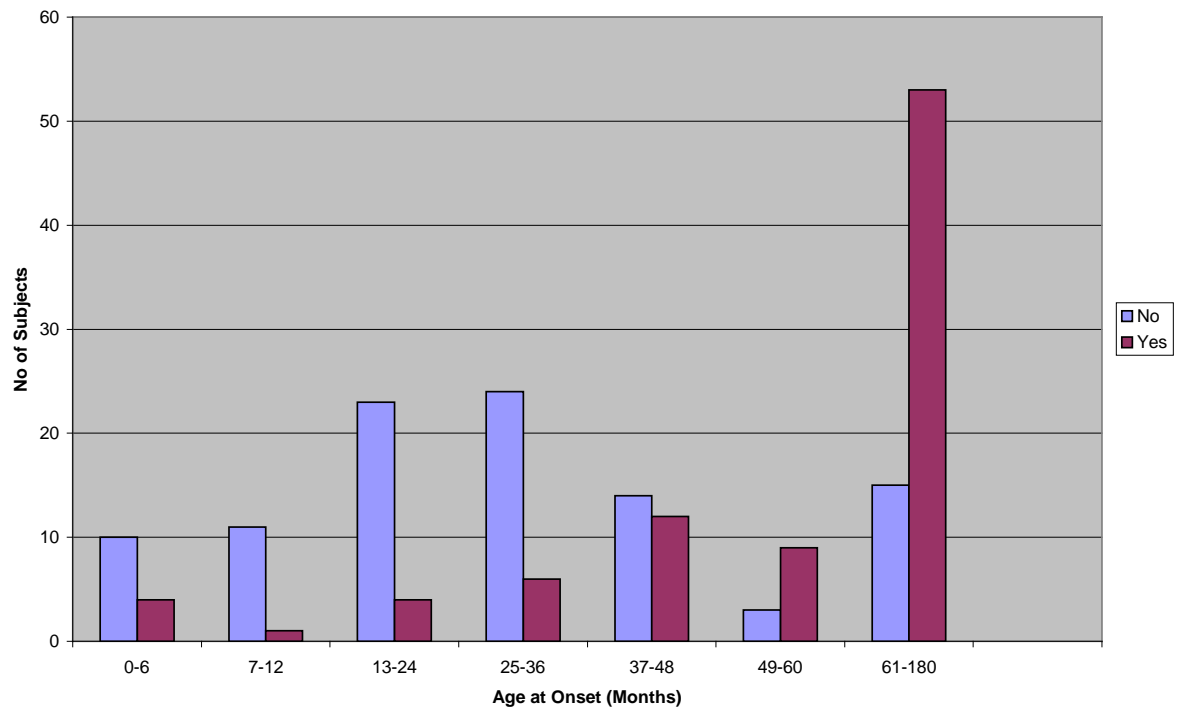
Figure 4.1.3. The age at onset of OM and the presence of hearing loss among subjects with ASOM

Table 4.1.3. PTA in decibel (dB) among cases of CSOM showing hearing loss (HL) and normal hearing

Normal (0–25dB)	Mild HL (26–45 dB)	Moderate HL (46–60dB)	Moderate–severe HL (61–75 dB)	Severe HL (76–90 dB)	Profound HL (<90 dB)	Total
100 (53%)	69 (37%)	14 (7%)	6 (3%)	0	0	189 (100%)

Table 4.1.4. Frequency of attacks of otitis media in 89 patients with hearing loss

Variables	No. of attacks			
	0–3 (*n*=126)	4–6 (*n*=40)	7–9 (*n*=16)	10–15 (*n*=7)
Hearing loss	54 (42.8%)	21 (52.5%)	14 (87.5%)	7 (0%)

Table 4.1.5. Correlation between hearing loss and risk factors

Variables		Age at onset (group)	Social class (group)	No. of attacks (group)	URTI	Hearing loss	No. of people in household
Age at onset	Pearson correlation	1.00	-0.04	0.09	-0.01	-0.04	0.01
	Sig. (2-tailed)	-	0.57	0.22	0.87	0.62	0.93
(group)	No.	189	185	189	189	189	189
Social class	Pearson correlation	-0.04	1.00	0.12	0.06	0.14*	0.23†
	Sig. (2-tailed	0.57	-	0.12	0.44	0.02	0.00
(group)	No.	185	285	185	285	285	285
No. of attacks	Pearson correlation	0.09	0.12	1.00	0.35†	0.07	0.11
	Sig. (2-tailed)	0.22	0.12	-	0	0.35	0.15
(group)	No.	189	185	189	189	189	189
	Pearson correlation	-0.01	0.05	0.35†	1.00	0.05	-0.10
	Sig. (2-tailed)	0.87	0.44	0	-	0.36	0.08
URTI	No.	189	285	189	289	289	289
Hearing loss	Pearson correlation	-0.04	0.14*	0.07	0.04	1.00	0.05
	Sig. (2-tailed)	0.62	0.02	0.35	0.36	-	0.41
	No.	189	285	189	289	289	289
	Pearson correlation	0.01	0.22†	0.11	-0.10	0.59	1.00
No. of people in	Sig. (2-tailed)	0.93	0	0.15	0.08	0.41	-
household	No.	189	285	189	289	289	289

Correlation significant at the 0.05 level (2-tailed); † correlation significant at the 0.01

level (2-tailed); sig., significance; URTI, upper respiratory tract infection.

NB: Controls had audiometry and are also included in social class, URTI, household

numbers.

4.2.   The Role of Neonatal Immunobiology in the Development of Early

   Suppurative Otitis Media

The subjects included 258 neonates, of which 186 were followed up for 1 – 1.25 years

for development of otitis media. After one year follow – up, at least 1 episode of EOM

was seen in 69 (37%); 38 purulent and 31 mucoid acute suppurative otitis media.

These were made up of 40 males and 29 females. The age at detection of SOM ranged

between 3weeks and 8 months, (mean $\pm$ SD = 14weeks $\pm$ 6. 2). All of them were on

breastfeeding at the time of follow – up. The gestational age at birth was between 31 -

43 weeks, (mean $\pm$ SD = 39 weeks $\pm$ 0.16), while the birth weight ranged between 1.25

– 4.3Kg, (mean $\pm$ SD = 3.10Kg $\pm$ 0.45). Out of 186, the mode of delivery was vaginal

in 162 while the rest were through the caesarean section. The values of the parameters

assayed from fetal serum at birth among the participants were Ig G 234.00 – 9346.00

mg/ml, retinol 0.40 - 1.80µg/L, Zinc 0.12 - 1.80µg/L and IFN ɤ 31.00 – 108.00pg/ml.

Among the (cases) subjects who developed ESOM and those who did not develop

ESOM (control), respectively, the mean $\pm$ SD of the cord blood levels were Ig G

1180.00mg/ml$\pm$ 6.3 and 1370.20 mg/ml $\pm$ 9.40, retinol 0.95$\pm$ 0.14 and 1.08 µg/L$\pm$

0.18, zinc 0.88µg/L $\pm$ 0.13 and 1.05 µg/L$\pm$ 0.24; and IFN ɤ 45.30pg/ml $\pm$ 4.20 and

70.20pg/ml$\pm$ 24.50. The estimated gestational ages were control 38.4weeks, EOM

38.8weeks while the fetal weight values were 3.06Kg and 3.14Kg among control and

cases.

Multiple logistic regression analysis of the mean values using unpaired t-test between

the subjects who developed early OM and control subjects revealed significant

difference in the cord blood levels of retinol (p = 0.01), zinc (p=0.01) and IFN ɤ

(p=0.01) while there was no difference in the levels of Ig G (p=0.46), fetal weight (p=0.30) and gestational age at birth p=0.20), Table 4.2.1.

Sociodemographic analysis revealed normal birth – weight (>2.50Kg) in 173 neonates while low birth weight (<2.50Kg) was seen in 13 neonates, 8 cases and 5 control subjects. Malnutrition was found in 26/69 of the ESOM subjects compared 6/117 control subjects. Socioeconomic class was low in 38/69, high in 31/69. The frequency of otorrhoea was 0 - 3 in 58 cases while it was > 4 in 11 cases. Congenital abnormalities were found in 2 neonates. The congenital abnormalities were cleft palate and encephalocoele. Multiple logistic regression showed that malnutrition p = 0.03) and indoor cooking (p=0.04) were significant risk factors, while family history of otitis media (p=0.20), exclusiveness of breast feeding (p=0.34), day care center attendance (p=0.08), number of siblings (p=0.06) and presence of congenital malformations (p=0.62) were not statistically significant risk factors, table 4.2.2.

Table 4.2.1. Multiple logistic regression analysis of the values of cord blood markers among cases and control subjects

VARIABLES	*CASE (n =69)*	*CONTROL(n =117)*	p value
	Range(mean)SD	*Range(mean)SD*	
RETINOL(µg/L)	0.40 -1.80(0.95) 0.14	0.56-1.42(1.08) 0.18	0.03
ZINC (µg/L)	0.12-1.80(0.88) 0.13	0.18-1.20(1.05) 0.24	0.01
IgG(mg/ml)	234 - 9346 (1180) 6.31	234-6538 (1370.2) 9.40	0.46
INTERFERON GAMMA(pg/ml)	12 – 126 (45.50) 4.22	29 – 126 (74.30) 24.50	0.01
BIRTH WEIGHT(Kg)	1.98 – 4.3(3.10) 0.40	1.25-4.3(3.06) 0.49	0.30
GESTATIONAL AGE(weeks)	34 - 42 (38.80) 1.67	31- 45 (38.40) 2.26	0.20

Table 4.2.1. Multiple logistic regression analysis of the values of cord blood markers among cases and control subjects

VARIABLES	p value
Clinical Malnutrition	0.03
Indoor cooking	0.04
Family history of otitis media	0.2
Day care center attendance	0.08
Number of siblings	0.06
Exclusiveness of breast feeding	0.34
Congenital malformations	0.62

4.3. Serum and Middle Ear Immunoglobulins in Acute and Chronic Suppurative Otitis Media

The participants were 399, comprising of 171 healthy control subjects and 228/284 acute OM subjects who had at least 10 months follow up. They were made of 232 males and 167 females, between the ages of 6 months and 9 years, (mean $\pm$ SD = 7 $\pm$ 2.32). The controls and subjects were comparable in age and sex as shown in table 4.3.1.

The culture of bacterial isolates revealed that organisms responsible for the ASOM were *Pseudomonas aeruginosa* in 36%, *Staphylococcus aureus* 36%, *Streptococcus spp* 31% and *Haemophyllus influenza* 29%. There were multiple isolates cultured in 56% and no organism was cultured in 6% of cases, the others are as shown in table 4.3.2.

Out of the 228 ASOM subjects, persistence of disease, CSOM was seen in 87 (46%) while the criteria for resolution of OM was met in 141 (61%). Among the 228 acute OM subjects, POM accounted for 126 (55%) and MOM were 102 (45%). Among the resolved ASOM subjects; there were 95 POM and 46 MOM, while the 87 CSOM subjects were made up of 31 POM and 56 MOM subjects (p= 0.002).

The mean serum levels of IgG were 1051mg/dL in normal healthy children (controls), 666.1mg/dL in ASOM and 1321.1mg/dL in CSOM, while the MES levels of ASOM and CSOM were 203.4 mg/dL and 511.5mg/dL respectively; Table 4.3.3. The mean serum levels of IgM were 35mg/dL in control, 64.10mg/dL in ASOM and 40mg/dL in CSOM, while the MES levels of IgM were ASOM 22.59mg/dL and CSOM

3.44mg/dL; Tables 4.3.3. The mean serum level of IgA were 36mg/dL in control, 46.1mg/dL in ASOM and 41mg/dL in CSOM, while the MES level of IgA were ASOM 228.30mg/dL and CSOM 85.40mg/dL, Table 4.3.3.

The mean MES : serum ratio for IgG were 0.3 and 0.4 in ASOM and CSOM respectively while for IgM it was 0.35 and 0.1 in ASOM and CSOM respectively.

The ratio of serum IgG level of control and ASOM was 0.66 while control and CSOM was 1.3. The mean MES: serum ratio for IgA was 4.95 for ASOM and 2.10 for CSOM. The ratio of serum IgM level of control and ASOM was 1.60 while control and CSOM was 0.88. The ratio of serum level of control to ASOM was 1.30 while control to CSOM is 1.14.

Multivariate analysis using unpaired t-test to compare the mean revealed significant difference between serum IgG level of ASOM and CSOM ($p= 0.043$) and MES IgG ($p=0.02$) in ASOM and CSOM; and between the MES IgA level of ASOM and CSOM ($p= 0.01$). However, there was no significant difference between serum IgG level in control and otitis media subjects (ASOM, $p=0.25$, CSOM, $p=0.46$), serum IgM level in control and CSOM ($p=0.62$) and IgM control and ASOM sera ($p=0.73$), MES IgM level of ASOM and CSOM sera ($p=0.06$), serum IgA level of ASOM and CSOM ($p=0.57$), and between the serum IgA level in control and otitis media subjects (ASOM, $p=0.25$, CSOM, $p=0.60$).

In further assessing the role of immunoglobulins in the outcome, the nature of the middle ear secretion was studied. The middle ear secretions encountered were sub-divided into mucoid secretion, mucoid otitis media (MOM) and purulent secretion, purulent otitis media, (POM). Comparing the serum Immunoglobulins between MOM and POM, it was found that the mean serum levels of IgG were 1785mg/dL in POM,

1302.5mg/dL in MOM,; while the serum IgA levels were 60.8mg/dL in POM and 88mg/dL in MOM and the IgE levels were 53 mg/dL, 331 mg/dL and 336 mg/dL respectively; Table 4.3.4.

Univariate analysis using unpaired t-test revealed significant difference in the serum IgG (p=0.019). While there were no significant statistical differences in the serum levels of IgA (p= 0.71) and IgE (p=0.95), although the mean values were higher in MOM than POM; Table 4.3.4.

The mean MES levels of IgG, A and E were 994.80mg/dL, 264.70mg/dL and 301mg/dL in POM, while in MOM, they were 833.90mg/dL, 132.20mg/dL and 439mg/dL; Table 4.3.5.

The univariate analysis revealed significant difference between POM than MOM in the MES levels of IgA (p=0.03) and IgG (p=0.01). However, there was no significant statistical difference in the MES level of IgE (p= 0.88); Table 4.3.5.

The MES/serum ratios of IgE were 0.89 and 1.30 among POM and MOM respectively (p=0.03). The serum IgA/IgG ratios were 0.03 and 0.07 among POM and MOM respectively, however, the IgA/ IgG ratio in the MES were higher than sera, 0.3 and 0.2 for POM and MOM respectively, Table 4.3.8. Comparing the middle ear response to serum, the MES/ serum ratio of Ig A/Ig G value was 0.3/0.03(10) for POM and 0.2/0.07(2.67) for MOM, (p = 0.001), this showed that middle ear immune response was significantly lower in MOM than POM, Table 4.3.6.

Table 4.3.1. The demographic distribution of subjects with ASOM and healthy control subjects

Variables	ASOM(n=228)	Control (n=171)
Male	137	95
Female	91	76
Age	6months–7years	7months–8years
	(mean$\pm$SD= 5.5years$\pm$2.3)	(mean$\pm$SD=6.1years$\pm$ 3.2)

Table 4.3.2.Bacterial isolate in acute suppurative otitis media.

n= 228

ORGANISM	NUMBER	PERCENTAGE(%)
Pseudomonas aeruginosa	82	36%
Staphylococcus aureus	82	36%
Streptococcus spp.	71	31%
Haemophyllus influenza	66	29%
Escherichia coli	25	11%
Klebsiella sp	18	8%
Staphylococcus epidermidis	18	8%
Candida spp	17	7%
Others	14	6%
No growth	14	6%

*There are mixed isolates

Table 4.3.3. Values of IgG, A and M level in serum and middle ear secretion

Ig (mg/dL)	Control Sera(n=171)	AOM Sera(n=141)	AOM MES(n=141)	COM Sera(n=87)	COM MES(n=87)
IgG					
Range	0-2802	0-1868	0 – 934	0 – 2802	0- 1868
Mean	1051.00	666.10	203.40	1321.10	511.50
Median	1354.00	600.00	135.00	934.00	327.00
SD	872.60	14.43	269.40	21.30	535.70
IgM					
Range	0-55	0 – 95	0- 104	0 - 90	0 – 55
Mean	35	64.10	22.59	40	3.44
Median	55	70	14.00	55	1.20
SD	27.75	31.21	37.32	36.20	13.75
IgA					
Range	0 - 61.50	0 – 45.50	130 – 326.3	0 – 55.1	55.0- 115
Mean	36	46.12	228.30	41	85.40
Median	31.50	42.60	192.00	40	81
SD	25.90	16.50	16.22	26.30	13.64

Table 4.3.4: Serum immunoglobulin level in Purulent OM, Mucoid OM subjects and healthy control

erum immunoglobulin (mg/dL)	Control (n=171) Range (mean) SD	PURULENT (n=126) Range(mean) SD	MUCOID (n=102) Range(mean) SD	p value
IgG	0-2802 (1051) 8.72	1468.90 - 2101.50 (1785.2) 26.4	1059.1- 1545.50 (1302.5) 21.70	0.02
IgA	0–120 (36) 3.71	29.4-92.2(60.8) 9.42	44.8-132.40 (88) 10.20	0.71
IgE	0-150 (53) 1.57	0-380 (331.50) 6.90	200-431.10 (336.00) 4.90	0.95

Table 4.3.5: Levels of middle ear secretion immunoglobulin in purulent and mucoid OM

MES Immunoglobulin	PURULENT(n=126) Range(mean) SD	MUCOID(n=102) Range(mean)SD	p-value
IgG (mg/dL)	617.80 - 1049.90 (994.80) 6.80	0-4000 (833.90) 11.40	0.03
IgA (mg/dL)	25.4-450 (264.70) 12.30	0 – 320 (132.20) 9.70	0.01
IgE (mg/dL)	0 – 900 (301.0) 31.70	0 – 900 (439.00) 42	0.88

Table 4.3.6. The ratio of IgA/IgG in MES and serum

IgA/ IgG ratio	Purulent	Mucoid	p value
MES	0.03	0.20	0.61
Serum	0.03	0.07	0.54
MES/serum	10.00	2.60	0.01

4.4.   Role of Elevated IgE in the course of Suppurative Otitis Media

The study comprised of 228 acute OM subjects made of 137 males and 91 females, between the ages of 0 and 9 years, mean of 7 years (SD =3.4). Out of these, there was chronicity in 87 (38%).

History of hypersensitivity and positive skin test to one or more of the allergens among dust, house dust mite, mould, cockroach and poultry feather was obtained from 15/71 (21%) controls and 105/228 (46%) subjects with SOM. These comprised of 66/87 (76%) CSOM and 39/141 (28%) resolved ASOM. The subjects were made of 59 males and 46 females while the controls were 38 males and 33 females, both were comparable in age, table 4.4.1.

The mean total IgE levels in sera were 52.1mg/dL in control, 63.9 mg/dL in ASOM and 79.2 mg/dL in CSOM while the MES levels were AOM 60.4 mg/dL and CSOM 102.0 mg/dL (table 4.4.2.) Comparing controls with ASOM and CSOM, the ratio of serum IgE level of control to ASOM was 1.22 and CSOM 1.5.

The mean serum IgE levels were 331 mg/dL and 336 mg/dL in POM and MOM respectively. The mean MES level was 301mg/dL and 439mg/dL in POM and MOM. The ratio of total IgE level in the MES to serum was CSOM was 1.40, about twice the ratio in ASOM which was 0.75. However, the MES/serum ratios of IgE were 0.89 and 1.30 among POM and MOM respectively (p=0.03).

Univariate analysis using unpaired t-test to compare the mean revealed significant difference between total IgE level of MES in acute and chronic otitis media (p= 0.04) but no correlation between total IgE level of control and ASOM sera (p=0.10), control and CSOM sera (p=0.7), AOM and CSOM sera (p=0.3).

Table 4.4.1. The demographic distribution of each study group

Variables	Subjects(n=105)		
	CSOM(n=66)	Resolved ASOM(n=39)	Control(n=71)
Male	41	18	38
Female	25	21	33
Age	1 – 9 years (mean $\pm$ SD = 7.20$\pm$3.40)	1 – 8years (mean $\pm$ SD = 5.5$\pm$3.20)	1–9years (mean $\pm$ SD = 6.50$\pm$3.60)

Table 4.4.2. The values of the IgE in serum and middle ear secretion

IgE (mg/dL)	Control Serum(n=71)	AOM Serum(n=39)	AOM MES(n=39)	COM Serum(n=66)	COM MES(n=66)
Range	0-150	0 – 250	0 – 250	0 - 550	0- 550
Mean	52.10	63.90	60.40	79.20	102.00
Median	37.50	59.60	47.00	62.40	40
Standard Deviation	1.57	4.23	6.14	1.64	3.63

4.5. Interferon Gamma in Suppurative Otitis Media

The study started with 358 ASOM subjects, 12 month follow-up was achieved in 304 subjects (85%). Of these, there was resolution of ASOM in 187, while there was persistence of otorrhoea beyond 3 months (CSOM) in 117. These were made of 173 males and 131 females, between the ages of 4 months and 9 years (mean $\pm$ SD = 6.6 years $\pm$ 1.32). The nature of otorrhoea in ASOM was purulent in 171 and mucoid in 133.

The range of MES IFN-γ in the ASOM subjects was 12 – 126pg/mL, the mean among those with resolved ASOM was 27.20pg/mL, (SD = 4.12) while among those who progressed to chronicity the mean was 73.10g/L (SD = 12.04); Table 4.5.1.

Among the purulent OM, the mean MES IFN-γ was 43.5, (SD 15.6) while among the mucoid OM it was 74.3 (SD=19.1), Table 4.5.2.

Univariate analysis using unpaired t - test to compare the mean IFN-γ revealed significant difference between resolved ASOM and CSOM (p=0.01) and between purulent OM and mucoid OM (p= 0.00). The age and sex distribution were not statistically significant between resolved ASOM and CSOM (Table 4.5.1) and between purulent and mucoid OM (Table 4.5.2). Pearson correlation test revealed significant reverse correlation of IFN-γ with MES Ig G (p=0.01), Ig E (p=0.03) and MES IgA (p=0.001), Table 4.5.3.

Table 4.5.1. Comparing the variables between Resolved ASOM and CSOM subjects

MES IFN-γ (pg/mL)	Resolved ASOM (n=187)	CSOM (n=117)	p values
(mean±SD)	27.20± 4.12	73.10± 12.04	0.01
Age(years)	6.50±2.30	6.90±2.60	0.92
Sex: Male(n=173)	112	61	
Female (n=131)	75	56	0.63
Purulent OM (n=171)	126	45	
Mucoid OM (n=133)	61	72	0.01

Table 4.5.2: Univariate analysis comparing the MES IFN-γ between purulent and mucoid OM

MES IFN-γ (pg/mL)	Purulent OM (n=171)	Mucoid OM (n=133)	p values
Mean±SD	43.50± 15.60	74.30 ±19.10	0.01
Age(Years)	6.20±3.60	6.8±3.20	0.87
Sex: Male(n=173)	106	67	
Female (n=131)	65	66	0.71
Resolved ASOM	126	61	
(n=187)			0.01
CSOM (n=117)	45	72	

Table 4.5.3. Univariate analysis between MES IFN-γ and Immunoglobulins

VARIABLE	Ig G	Ig A	Ig E
MES IFN-γ	-0.20	-0.32	-0.13
	0.01	0.00	0.03

4.6. The Role of Nutritional Factors in the Aetiology and Outcome of Suppurative Otitis Media

The participants were 399, comprised of 171 healthy controls and 228/284 acute OM subjects who had at least 6 month follow up. They were made of 232 males and 167 females, between the ages of 6 months and 9 years, (mean $\pm$ SD =7 $\pm$ 2.32). The control and subjects were comparable in age and sex as shown in table 4.6.1. Among the 228 acute OM subjects, POM accounted for 126 (55%) and MOM were 102(45%). The criteria for resolution of OM was met in 141 (61%) subjects; 95 POM and 46 MOM, while chronicity was seen in 87 (46%), 31/126 POM and 56/102 MOM (P= 0.02).

The range of serum retinol in the AOM subjects was 1.61 – 2.63µg/L, mean of 2.07µg/L and median value of 2.09 µg/L (SD=0.12). Among control subjects, the range was 2.50µg/L - 2.80µg/L, mean of 2.58µg/L and median value of 2.61µg/L, (SD = 0.14). While the CSOM subjects ranged between 0.80µg/L – 2.86µg/L, mean of 1.58 µg/L and median value of 1.28µg/L, (SD=0.48).(Table 4.6.1 and Table 4.6.2). The mean serum retinol in POM and MOM were 1.92µg/L and 1.40µg/L respectively.

Univariate analysis using unpaired t-test to compare the mean serum retinol revealed significant difference between ASOM and control (p= *0.01*) and CSOM (p=*0.01*); and between POM and MOM (p= 0.03).

The range of serum zinc was 0.50 -1.20µg/L, with a mean of 1.06 µg/L and 1.07µg/L among resolved ASOM and CSOM, (Table 4.6.3). The mean serum zinc levels were 1.50µg/L, 1.12µg/L among POM and MOM respectively.

Univariate analysis using unpaired t-test to compare the POM and MOM revealed significant difference in the serum Zinc (p=0.01), Table 4.6.4. However the difference was not significant between resolved ASOM and CSOM (p = 0.76).

Table 4.6.1. Univariate Analysis comparing serum retinol level (µg/L) between AOM and normal healthy control.

Group	No	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]
ASOM	264	1.53	0.05	0.58	1.43 - 1.64
Control	52	2.61	0.04	0.29	2.53 - 2.69

P > |t| = 0.01, showing that serum retinol is protective.

Table 4.6.2. Univariate Analysis comparing serum retinol level (μg/L) between resolved AOM and COM

Group	N	mean	p50	SD	min	max
Resolved ASOM	148	2.08	2.09	0.14	1.611	2.64
CSOM	116	1.63	1.28	0.12	0.83	2.86

P > |t| = 0.01, showing that serum retinol is protective

Table 4.6.3. Univariate Analysis comparing serum Zinc level (µg/L) between resolved AOM and COM

Group	N	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]
Resolved ASOM	148	1.07	0.02	0.24	1.02 - 1.11
CSOM	116	1.06	0.02	0.25	1.01 - 1.10

p = 0.76

Table 4.6.4: Univariate Analysis comparing serum Zinc level (µg/L) between POM and MOM

Group	N	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]
POM	148	1.5	0.02	0.14	1.02 - 1.11
MOM	116	1.12	0.02	0.18	1.01 - 1.10

P = 0.01, showing that a lower serum retinol is predictive of development of MOM

CHAPTER 5

5.0   DISCUSSION

5.1.   Epidemiologic risk factors of early onset otitis media

The main finding was that 70% of children with CSOM had had first episode of otitis media within the first year of life. In addition, allergy, low social class, indoor cooking and living in congested environment constituted major odds for the development of CSOM. The preponderance of early onset otitis media among children with CSOM in this survey is comparable to the other works (Merchant *et al.,* 1984; Clyde *et al.,* 1997; Robert *et al.,* 2002) which reported 63 – 94%. This informed the search for additional risk factors which might further predispose to early OM. Further significance is the association of early otitis media and hearing loss. This could be a reflection of the severity of the disease in infancy, leading to rapid destruction of ossicles, middle ear mucosa and fibrosis with hearing loss; the danger of this is the resulting impairment of receptive language and verbal aspects of cognition (Friel – Patti and Finitzo, 1990; Paradise *et al.,* 2000).

Children who experienced otitis media (OM) onset in the first few months of life were at greater risk of chronic otitis media with effusion and recurrent OM than children who had later onset (Teele *et al.,* 1989; Bluestone, 2004; Pettigrew *et al.,* 2004; Karevold *et al.,* 2006; Daly *et al.,* 2007). The contributory factors included immature immune system and anatomic factors. However, prenatal, early environmental exposures and poverty have also been reported. The twin and triplet study of Casselbrant *et al.* (1999) on OM suggested a strong genetic component to the onset and frequencies of episodes of middle ear effusion and AOM in children. The prevalence of early onset otitis media in children within the first year of life according to reports ranged between 39% - 91% (Owen *et al.,* 1993; Dagan *et al.,* 1996;

Rossenfeld *et al.,* 2005). The works on early OM are few, particularly so in the sub Saharan Africa, most have focused on school age.

This study was carried out in a sub-urban hospital setting with predominant low socioeconomic class, while most of the other studies were prospective search in group daycare program. In the current study, nasal allergy, low socioeconomic status and increased number of children in household have been shown to be significant risk factors for early OM compared to later onset. This could be due to early exposure to overcrowding with inadequate ventilation and high humidity, lower ciliary function, change of nasopharyngeal flora and recurrent respiratory infection.

Adults and children of lower socioeconomic status (SES) were at higher risk for a wide range of communicable infectious diseases, especially respiratory infections and other stresses (Cohen *et al.,* 2006). Similarly, studies from India, China, Britain and United State of America have reported that low income and poor living conditions were associated with greater incidence of acute upper respiratory tract infections and many more missed days of school and more days in bed as a result of acute respiratory illnesses and otitis media (Sim *et al.,* 1976; Egbuonu, 1982; Ana *et al.,* 1990; Cruiz *et al.,* 1990; Power, 1992; Paradise *et al.,* 1997; Deb, 1998).

Why are people with lower SES at greater risk for infectious illness? There were two categories of explanation. One category attributed greater incidence to increased exposure to infectious agents with decreased socio-economic status. Families within the lower socio-economic class often have more children and live in more crowded quarters; both environmental conditions conducive to transmission of infectious agents (Sim *et al.,* 1976; Taber *et al.,* 1981). Poor environmental sanitation and poor hygienic practices might also increase exposure among poorer and less educated groups.

Alternatively; socio-economic class may increase risk of infection and infectious illness because it alters the body's ability to fight off infection. For example, those with lower SES might lack information about vaccination, lack access to medical care, or be unable to afford vaccinations (Solbera *et al.,* 1997). Vaccinations boost the immune system's ability to respond to specific infectious agents and hence reduce incidence and severity of illness. Inadequate nutrition among lower SES groups may also contribute to poorer host resistance. Malnutrition is known to suppress the immune system's ability to fight off infections and has been identified as a pathway linking poor children to disease risk (Deb, 1998). Health practices that worsen with decreasing education are also thought to act as pathways linking SES to infectious susceptibility. For example, greater rates of smoking contributed to greater susceptibility to respiratory infectious illness among teenagers and adults (Cohen *et al.,* 1997; Paradise *et al.,* 1997); whereas passive smoke exposure increased susceptibility among children (Graham, 1990). The other health practices associated with increased risk of respiratory infection such as inadequate physical exercise and poor sleep quality are also more prevalent among those lower on the SES gradient (Cohen *et al.,* 1997; Cohen *et al.,* 1999).

Studies (Graham, 1990; Cohen *et al.,* 1997; Cohen *et al.,* 1999) have found association between unemployment and susceptibility to clinical colds. The major outcome in the work of Cohen *et al.* (1999) was that after accounting for all of these variables, those who were unemployed were 3.4 times ($p < 0.03$) more likely to develop colds than the remainder of the volunteers. The analyses indicated that at least part of the association could be attributed to unemployed people smoking, having poorer sleep quality, and elevated levels of nor-epinephrine.

In this study, allergy has emerged a significant risk factor for early development of OM. Similarly, Auinger *et al.* (2003) and Karevold *et al.* (2006) also identified an

increase in allergic and atopic conditions as risk factors for the increased prevalence of OM in children. This could be explained by nasal allergy with eustachian tube dysfunction and middle ear inflammation, however, direct middle ear allergy has also been proposed. The bar chart of frequency of recurrence of OM in this study showed greater frequency in the early onset than later onset group. Of the 132 children with early otitis media 55 had more than 6 episodes compared with 7/35 of those with later onset otitis media, although this did not show significant statistical correlation. In other studies (Teele *et al.,* 1989; Owen *et al.,* 1993; Dagan *et al.,* 1996; Casselbrant *et al.,* 1999; Rossenfeld *et al.,* 2005), significant correlations were found between early onset OM and increased risk of recurrence of otorrhoea. In the report of Homoe *et al.* (1999) children with recurrent OM had their first AOM episode at a significantly younger age than children with < 5 AOM episodes and 83% of children with recurrent acute OM had their first acute OM episode before 12 months of age compared with 53% of children with < 5 episodes(P <0.05). Faden *et al.* (1997) had reported that most infants were colonized with OM pathogens such as *Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis* by age 6 months and colonization was more frequent during episodes of upper respiratory illness. An immature immune system, anatomic factors, environmental factors and prenatal exposures to low dietary vitamin C intake have been reported to be significantly related to early AOM (Dagan *et al.,* 1996; Daly *et al.,* 1999). This gave the impression that the mechanism of prenatal influence might be expressed through maternal undernutrition particularly in the trace elements and overwhelming viraemia passed down to the infant at birth and predisposing to OM. All of these might form the thrust of future research into the risk and pathogenesis of early onset otitis media which is important in view of the high prevalence of early OM and its significant association with hearing loss.

5.2. Hearing Loss And Otitis Media

This study has shown that the prevalence of hearing loss to be 47% among subjects with CSOM. The hearing loss is predominantly mild, however, moderate to moderate-severe hearing loss seen in about 10% may result in significant impairment which may require medical intervention. Chronic suppurative otitis media (CSOM) is a major cause of acquired hearing impairment in children, especially in developing countries. Most approaches to treatment have been unsatisfactory or are very expensive and difficult; for example parenteral aminoglycosides require long hospitalization and are potentially ototoxic. The hearing impairment produced by otitis media affects intellectual performance, which has been demonstrated by several studies (WHO, 2004). Lack of access to hearing aids aggravates the hearing disabilities. At a recent WHO meeting of experts from 15 African countries, CSOM was considered the most common cause of persistent mild to moderate hearing impairment among children and young people in developing countries (Bluestone *et al.,* 2002). In Nairobi, Kenya, hearing loss was found in 64% of school children with CSOM and in only 3.4% of children without CSOM (Bluestone *et al.,* 2002).

The high prevalence of hearing loss in CSOM as found in this study compared with reports of 50% from some developed countries (Bluestone *et al.,* 2002; Olusanya *et al.,* 2004). These figures were higher than reports from developing countries of prevalence rates of hearing loss following OM with effusion of 13.8–36.2% (Jacob *et al.,* 1997; Saim *et al.,* 1997; Daly *et al.,* 1999; Olusanya *et al.,* 2004). This could be due to the fact that in developing countries, CSOM is often reported to be more common and more easily detected than OM with effusion (Daly *et al.,* 1999; Bluestone *et al.,* 2002; Olusanya *et al.,* 2004). Fortunately, the hearing loss was predominantly conductive and in the majority was mild without functional impairment, hence not requiring

further management. In this study, the 3-month cut-off was taken as the reference for selecting ASOM and CSOM, similar to studies in the USA, although the WHO definition of CSOM is 2 weeks aural discharge associated with perforation (WHO, 1998). The sequelae of CSOM, such as fibrosis within the middle ear, ossicular erosion, ankylosis of the ossicular joints and labyrinthitis due to diffusion of toxins and bacterial breakdown products, have been reported as the pathogenesis of deafness in these patients. The potential deleterious effects of moderate-to-severe conductive deafness and SHL on language and education make it imperative to implement early management of CSOM. Clinical and histopathological evidence have linked SHL in CSOM to entry of toxic materials through the round-window membrane into the inner ear, leading to biochemical alteration of the inner-ear fluids, serofibrinous precipitates and inflammatory cells in the scala tympani, all of which result in gradual end-organ dysfunction and accentuated threshold shift (Paparella *et al.,* 1970; English *et al.,* 1973; Beales, 1979; More and Best, 1980; Paparella, 1981; Papp *et al.,* 2003; Kirtane *et al.,* 2007). Similar to other reports (Paparella *et al.,* 1970; Papp *et al.,* 2003), the mean duration of CSOM is greater in patients with SHL. This suggested that the severity of hearing loss might be associated with increasing duration of the disease (Paparella *et al.,* 1970; Papp *et al.,* 2003; Feng and Chen, 2004), although the association between duration of disease and degree of SHL has not been confirmed by all (Feng and Chen, 2004).

In this study, socio-economic status was the only risk factor which showed a statistically significant association with development of hearing loss. This is similar to reports from Britain (Macandie, 1999) and the USA (Stahlberg, 1986; Paradise *et al.,* 1997; Cohen, 1999) of greater severity of OM in children from lower social classes with less formal education and parents who are unemployed. The contribution of low

socio-economic status to increased severity of OM might be multifactorial. Families of a lower social class often have more children and live in more congested homes with poor sanitation and hygiene, all of which create environmental conditions conducive to transmission of infectious agents (Macandie, 1999; Feng and Chen, 2004). In addition, malnutrition, which commonly accompanies low socio-economic status, suppresses the immune system and places poor children at greater risk of disease (Paparella *et al.,* 1970; Sims *et al.,* 1976; Beales *et al.,* 1979; Cohen, 1999) Such children also have diminished access to appropriate health services and immunization.

Although causality cannot be inferred from these data, the accuracy of the parents' recollection of the number of attacks of OM could also be a limitation to this study. However, the findings suggest a need for more basic research on the social correlates of CSOM and the need to identify the impact of socio-economic and nutritional status on hearing outcome and the course of disease.

5.3.   The Role of Neonatal Immunobiology in the Development of Early

Suppurative Otitis Media

The finding of this study identified defective type 1 immune response in neonates with early suppurative otitis media. This is evidenced by the finding of low cord blood levels of IFN γ which showed to be significant factors in the development of OM within the first year of life. IFN γ is produced by the T – lymphocytes in response to viral or bacterial challenge. Immature perinatal immune system and oxidative stress have been recognized as important contributing factors to the overall incidence of otitis media (OM) in infancy (Castro and Freeman, 2001). According to Facione. (1990) and Kemp. (1990) the first episode of otitis media occurred about six months of age, highest between seven months and three years of age, coinciding with the loss of

maternal antibody protection. The development of OM was thought to have its origins in early infancy, a period when the newborn's inexperienced immune system is maturing to develop an effective protection against pathogens and achieve immunological tolerance towards harmless antigens (Facione, 1990; Kemp, 1990; Daly *et a*l., 1999). Research has focused on the prenatal and neonatal periods to identify factors that increased the risk of non – communicable diseases (Bluestone, 2004; Pettigrew, 2004; Daly *et al.,* 2007). However, little research has been devoted to the role of neonatal immunobiology in the aetiology of early OM, although OM incidence is highest in the first 2 years of life and it is the most commonly diagnosed childhood disease (Daly *et al.,* 1999; Lasisi *et al.,* 2008).

This is significant because the onset of OM in the first few months of life may lead to a greater risk of chronic otitis media with effusion and recurrent OM than children who have later onset (Daly *et al.,* 1999; Pettigrew, 2004; Daly *et al.,* 2007). This study had documented the incidence of OM per livebirth in the first year of life which was 37%. The incidence appeared within the range of previous studies which reported between 14.8% and 73% (Berman, 1978; Lasisi *et al.,* 2007). This is the first prospective follow – up report from the sub - Saharan Africa hence the impression that it could be useful for planning in our region. The protocol in this study involved telephone calls to the various homes of participants and clinic visits, thus reducing proportion of missed cases to an insignificant level.

IFN γ is produced by the T – lymphocytes in response to viral or bacterial challenge. The main biological activity appears to be immunomodulatory, modulate T-cell growth and functional differentiation (Fischer *et al.,* 1999). It is a growth-promoting factor for T-lymphocytes and potentiates the response of these cells to mitogens or

growth factors (Spurzem, 1996; Sands, 1999; Yetiser, 2002; Neaville, 2003). In addition, it has been shown to activate macrophages, stimulate cytotoxic cell activity and increase B – cell differentiation and antibody production (Sands, 1999; Fischer *et al.,* 1999; Yetiser *et al.,* 2002; Neaville, 2003). IFN γ is involved also in processes of bone growth and inhibits bone resorption probably by partial inhibition of the formation of osteoclasts (Twomey *et al.,* 1990; Ferro *et al.,* 1993; Serfilippi *et al.,* 1994; Spurzem *et al.,* 1996).

Treatment with IFN γ of patients with systemic inflammatory response syndrome characterized by an impaired function of monocytes has been shown to augment functions of monocytes and to result in clearance of sepsis in the majority of these patients (Twomey *et al.,* 1990; Weiner, 1991; Farrar and Schreiber, 1993). The impression from this study is that these immunoprotective roles of IFN γ are deficient from neonatal life in those who had low serum level, hence their susceptibility to development of EOM among other infections. The interferon Gamma in cord blood may be passed from the mother during intrauterine life or a result of the body response to ongoing infections or both. These infections may be newly acquired in the neonatal life or a result of intrauterine exposure.

In this study, maternal serum level of these markers was not measured because we were not looking into the effect of these on the neonates. In addition, this study did not do a serial measurement of these markers because the study was studying the effect of the baseline levels of these markers on the development of ESOM. However, this study has considered the effect of breastfeeding, as all of the subjects were breastfeeding during the study, although no weaning method was recommended. The mothers were practicing the traditional method of weaning common to the

environment; all the mothers practiced breastfeeding exclusively for the first 6 months. However, it may be difficult to rule out the exclusiveness of the practice.

The variety of protective factors in human milk may compensate for the immaturity of the infant's immune system and the low production of the defensive agents in mucosal secretions of the newborn infants (Walker, 2004; Newburg, 2005). It contains immunoglobulins, lactoferrin, lysozyme, antiviral lipids and oligosaccharides) and interleukins, transforming growth factor, antioxidants such as vitamins A, C, and E, hormones and growth – promoting factors (Hammosh, 2001; Cummings *et al.,* 2002; Walker, 2004; Newburg, 2005). In addition to meeting the infant's energy and nutrient needs, the human milk provides an immunological support system extending from the mother to the infant during the first few months of life (Prasad et al., 2007).

The gestational age and birth-weight were not found to be significant factors in the development of ESOM. This suggested that there might be subtle difference between immunologic competence and the chronological maturity of a neonate. The neonates who developed ESOM were from different socioeconomic classes hence suggesting that environmental epidemiological factors may play confounding roles in development of infections. It was concluded from this study that ESOM might be the result of interplay between quantitative defect in the neonatal immunobiologic factors and the socioepidemiological variables. The study continues in order to determine further the significance of these factors.

5.4.   Serum and Middle Ear Immunoglobulins in Suppurative Otitis Media

The major findings from this study were the followings: Reduced serum and middle ear secretion of IgA in CSOM compared to ASOM and the significant difference between the MES levels in ASOM and CSOM (P= 0.0056). This suggested that

reduced IgA secretion from the middle ear may predispose to chronicity. Secondly, persistent secretion of IgG in the serum and middle ear were greater in CSOM subjects than ASOM. This was inferred from the ratio of serum level of CSOM to control >1 compared with ASOM to control <1 and correlation (P= 0.043) between serum IgG level of ASOM and CSOM. Jónsson *et al.* (2005) have reported that sustained low levels of IgA proved the strongest single indicator of susceptibility for recurrent otitis media (P=0.008) and respiratory tract infections (P=0.02), confirming our finding of low MES Ig A in CSOM compared to ASOM.

However, the range of MES/serum IgA ratio of 2.1 - 4.95 in ASOM and CSOM showed higher concentration of IgA in the middle ear than serum. In this study the middle ear immune response was based on the MES: Serum ratio of IgA / IgG index. This finding of an exaggerated middle ear concentration in both acute and chronic SOM in this study suggest that IgA production possibly originates from middle ear mucosa secretory response to inflammatory stimuli. The occurrence of IgA antibody in MES and its absence or gross reduction in simultaneously drawn serum has also been reported as an indicator of local antibody production (Soltan and Jenkin, 1983; Nasrat *et al.,* 1992; Ishizaka *et al.,* 1994). Of the 401 assays performed in their study, 41 instances of IgA antibody exclusively in MES were found.

The normal middle - ear mucosa appears to be devoid of any organized lymphoid follicles. However, expression of local immune responses has been observed in the middle ear mucosal epithelium during otitis media similar to other sites of common mucosal immune system. The immunological defense in the middle ear depends primarily on secretory antibodies, the eustachian tube factor and heredity (Murphy and Kyungcheol, 1997; Ogra, 1997; Faden, 2001). Recent studies on otitis media have provided more information on the development of immunologic reactivity and

characterization of the important components of the mucosa-associated lymphoid tissue and mucosal cytokines (Jung, 1988; Juhn *et al.,* 1997; Ogra, 1989). The production of specific secretory IgA by the adenoid and middle ear mucosa constituted an important part of the local immunological factor protecting the middle ear against invasion of both viruses and bacteria pathogens (Bernstein, 1999). IgA provided the dominant surface response to polysaccharide and lipopolysaccharide antigens but once the mucosa has been breached, most protection is provided by IgG2. It has an important role as a neutralizing antibody in the prevention of extensive tissue damage by this destructive toxin of a common respiratory pathogen (Sakamoto *et al.,* 1998; Bernstein, 1999; Drake –Lee *et a*l., 2003).

Bernstein. (1992) and others (Paton *et al.,* 1993; Virolainen *et al.,* 1995; Drake – Lee *et al.,* 2003) reported that IgA is produced in the adenoid and nasopharynx and directed against both viruses and bacteria pathogens in a genetically controlled fashion. Pichichero *et al.* (1981) and Paton *et al.* (1993) also found IgA class antibodies to the capsular polysaccharides of *Strep. pneumoniae* were detected more often in the middle ear and occurred independently of IgA antibody in serum. They also found correlation with the presence of the secretory component in pneumococcal antibody, indicating local production of IgA antibodies. Children with *pneumococci* found in MES samples developed nasopharyngeal IgA antibody responses to capsular polysaccharides more often than did children with *pneumococci* found only in the nasopharynx or not at all, indicating that the presence of *S. pneumoniae* in the middle ear was stimulus for nasopharyngeal antibody production. Similarly, Virolainen *et al.* (1994) and Virolainen *et al.* (1995) studied nasopharyngeal aspirate samples of 120 children with acute otitis media and detected IgA class antibody in 93%.

In this study, middle ear secretion samples was used for the estimation of the immunoglobulin similar to the work of Virolainen *et al.* (1994) and Virolainen *et al.* (1995). The finding of IgA in the middle ear secretion might suggest responses to nasopharyngeal and possible middle ear inflammation, associated with ASOM and CSOM.

The other immunoglobulin which this study also found significant in monitoring clinical outcome was serum IgG. The presence of pneumococcal antibody and immunoglobulin G in middle-ear effusion in the course of disease has been associated with rapid resolution (Sloyer *et al.,* 1974; Harada *et al.,* 1993; Wright *et al.,* 2000). However, there appeared to be persistence of disease despite persistent elevated immunoglobulin. The secreted IgG might be evidence of immunological reaction to particular strains of bacteria which might not be related to the current ear infection causing discharge. Faden *et al.* (1997) demonstrated that children develop serum bactericidal antibody to the infecting strain of nontypable *Haemophyllus influenzae* (NTHI) following otitis media. The presence of bactericidal antibody to a strain of NTHI was associated with protection from infection by that particular strain. Children who experience recurrent episodes have persisting serum bactericidal antibody to their original strain, but lack bactericidal antibody to the new strain. A serum bactericidal antibody response then occurs to the new strain following infection indicating that children develop a protective immune response following otitis media (Sakamoto *et al.,* 1998).

Barenkamp. (1986) and Barenkamp *et al.* (2001) observed that passive immunization with immune serum is protective in experimental NTHI otitis media and that bacterial outer membrane proteins may be the principal targets of protective antibody.

Of particular interest is also the origin of these antibodies. The serum – to - MES ratio of IgG and IgM of about 0.1 – 0.4 appear to support the view that antibody detected in the middle ear often reflects passive transfer from serum rather than local production (Pichichero *et al.,* 1981; Paton *et al.,* 1993; Virolainen *et al.,* 1995; Prasad *et al.,* 2007). Faden *et al.* (1997) and others (Paton *et al.,* 1993; Virolainen *et al.,* 1994; Virolainen *et al.,* 1995) suggested that the antibodies diffuse into the middle-ear space passively due to the acute inflammatory response.

However, Sloyer *et al.* (1977) reported that the fluid accumulation in the middle ear cavity represents, at least in part, a secretory immune response to infection of respiratory mucosa rather than a simple transudation. Although they were not conclusive on the different components of the middle ear secretion, findings from this study suggested that IgG and IgM in the middle ear might be a result of diffusion into the cavity following a response to the infective process.

It was concluded that middle ear IgA secretion was higher in ASOM than CSOM, thus reduced secretion might be associated with chronicity. The monitoring IgA assay might be useful index of determining likely progression to chronicity.

It is thus the impression that, the possible use of the serum immunoglobulin G be used as index of chronicity in OM patients. The monitoring of this immunoglobulin in AOM might help in determining the group of patient likely to progress to chronicity. This observation is expected to have important implications in understanding the immune response of children who experience recurrent episodes of otitis media.

5.5. Middle Ear Immune Response between Mucoid and Purulent Otitis Medi

The main finding from this study was that POM was associated with both higher MES levels of IgA and IgG and a greater fraction of MES/serum IgA secretory response

than MOM. These findings suggested that the middle ear immune response is greater in POM patients and it may be a major factor contributing to the difference in the nature of otorrhoea in OM.

The unifying feature of otitis media (OM) is any structural change in the middle ear associated with a permanent defect in the tympanic membrane. Usually, there is associated inflammatory mucosal disease in the middle ear, with or without the mastoid cells (Levenson *et al.,* 1989; Gilles and Asher, 1991; Chonmaitree *et al.,* 1992). The various predispositions have been documented, although the risk factors for OM favour the development by weakening of the immunological defence, increasing the inoculums and middle ear invasion by pathogens (Paparella *et al.,* 1990; Barbour, 1996; Chung *et al.,* 2002; Amirzargar *et al.,* 2003).

These different types of middle ear effusions and the clinical manifestations with which they are associated have been hypothesized to represent the typical inflammatory response (Paparella *et al.,* 1990; Chung *et al.,* 2002). In addition, various workers have reported differences in the middle ear immune response (MER) between mucoid and serous OM (Paparella *et al.,* 1990; Chung *et al.,* 2002).

Clinical observation in the outpatient has shown that purulent (POM) and mucoid (MOM) OM are the predominant types seen among our patients. The predominant immunoglobulin produced by mucosal tissues is IgA, a molecule that participates in host defense by inhibiting microbial adherence and invasion, inactivating bacterial toxins, and mediating cytotoxicity (Brandtzaeg *et al.,* 1992; Kilian *et al.,* 1996). In order to colonize the human respiratory mucosa, invading pathogens must overcome these protective effects of IgA. Several bacterial species elaborate extracellular endopeptidases, which cleave the hinge region of the serum and secretory forms of

IgA1 and release the antigen-binding Fab domains from the Fc portion of the molecule. As a result of cleavage, the agglutination activities of both free and antigen-bound IgA1 are eliminated (St Geme, 1990; Brandtzaeg *et al.,* 1992; Kilian *et al.,* 1996).

The IgA/IgG has been reported as the reliable measure of middle ear response in OM. In an assay of 32 sera and 50 MES of patients with MOM, Amirzargar *et al.* (2003) reported a higher ratio in the middle ear (0.23 - 0.35) than serum (0.13). This is similar to the report of Jeep. (1990) and Faden et al. (1989) and it is comparable with our finding of 0.2 - 0.3, corroborating the hypothesis that there is an independent immune response in the middle ear.

Emonts *et al.* (2007) have also shown that genetic variation in innate immune response genes such as *TNFA*-863A, *TNFA*-376G, *TNFA*-238G, *IL10*-1082 A, and IL6-174G alleles in the promoter sequences may result in altered cytokine production affecting TNFA (tumor necrosis factor A), IL1B (interleukin 1B), and IL6. This cytokine dysregulation have been associated with meningococcal infection. Increased expression of TNF-α, IL-1β, IL-6, and IL-10 have been observed during experimental otitis media in animals to contributes to an otitis-prone condition. (Alho *et al* 2003, Emont *et al.,* 2003; Long *et al.,* 2003; Melhus and Ryan, 2000).

This study also revealed that chronicity of OM had significantly higher association with MOM than POM. This might be explained by otitis media with effusion of our findings. Firstly increased middle ear response in POM compared to MOM, as found in this study, may encourage healing and recovery. This was revealed by MES/serum ratio of IgA/IgG which was 10 in POM compared to 2.6 in MOM. In addition, there was a higher proportion of allergy in MOM than POM evidenced by higher level of

serum IgE in MOM than POM. Furthermore, the MES/serum of IgE was less (0.89) in POM than MOM with significant statistical difference (0.03), suggesting greater role for allergy in MOM compared to POM. Allergic rhinitis patients have a higher risk of eustachian tube dysfunction, particularly during childhood. As a result of this dysfunction, the tympanic cavity is affected by: accumulated secretion, proliferation of microorganisms and changes of the hypersecretion of the lining mucous. The long-term persistence of these secretions may damage the normal functions of the ossicular chain and thus the transmission of sounds, leading to the onset of hypoacusis which may interfere negatively with the child's development. IgE sensitization and respiratory allergy symptoms are independent risk factors for the development of OME, suggesting that both immunological and mechanical pathways may contribute to the development of the disease. Additionally, they are suggestive of an interaction of allergy and viral infections in the upper airways, leading to an increased risk of MES (Yoon *et al.,* 1990; Alles *et al.,* 2001; Sobol *et al.,* 2002; Lazo-Saenz *et al.,* 2005; Chantzi *et al.,* 2006).

Comparisons of morphologic analysis in different otitis media types have shown that acute inflammatory changes were usually seen in purulent otitis media and chronic inflammatory changes were more severe in mucoid otitis media. However, there were overlaps in the histopathologic findings between different types suggesting a continuum of otitis media types, with one type of otitis media changing into another type (Yoon *et al.,* 1990). The persistence of mucoid otitis media has also been attributed to the cycle of events resulting from influx of neutrophil with release of neutrophil elastase which cause damage to middle ear epithelium. The damage stimulates production of mucus which impairs opsono-phagocytosis, and attracts more neutrophil by inducing epithelial cell production of IL-8. Ultimately mucociliary

clearance is impaired, allowing persistence and replication of colonizing bacteria and further influx of inflammatory cells (Brown *et al.,* 1985).

Furthermore, low serum zinc seen in MOM may be evidence of malnutrition which may potentiate chronicity. Dietary Zinc deficiency impairs overall immune function and resistance to infection by suppressing thymic function, T-lymphocyte development, lympho-proliferation, and T-cell-dependent B-cell functions (Shankar *et al.,* 1998).

The main addition to the literature from this study is the difference in the middle ear response and serum Zinc between POM and MOM and chronicity, which has not been previously documented. There is need for further studies on the significance of this difference in relation to the development of acute complications of OM, which have been found to be common in the developing countries. In this study, 6 month follow up was achieved in 228/284 AOM (80%) and the laboratory technique used in zinc and immunoglobulin assay were standard, although, total immunoglobulin was assayed. However, assaying for specific immunoglobulin need to be considered in future, as this may further substantiate the result.

It was therefore concluded, from this study, that middle ear immune response as measured by IgA/ IgG ratio in the MES and serum is greater in POM than MOM; in addition lower serum Zinc and high MES IgE were more common with MOM than POM and there was greater association of chronicity with MOM than POM

5.6.   Role of Elevated Immunoglobulin E Levels in Suppurative Otitis Media

In this study, the finding of ratio of total IgE in the MES to serum of 0.75 and 1.4 for AOM and CSOM respectively suggested that the IgE was secreted locally from the middle ear mucosa. In addition, the significant difference between the MES IgE level in acute and chronic otitis media, seemed to suggest that CSOM was associated with the exaggerated middle ear secretion of IgE. Hurst *et al.* (1999) reported elevated effusion concentrations of specific IgE in 83.3% of SOM patients and normal serum IgE in 30% of SOM. In a study of 88 children, Chantzi *et al.* (2006) concluded that IgE sensitization and respiratory allergy symptoms were independent risk factors for otitis media. Bernstein. (1992) and Bernstein and Doyle. (1994) studied 100 young patients with recurrent otitis media with effusion (OME), 35 of who had IgE-mediated nasal allergy. The allergic patients had higher levels of IgE in their serum and middle ear effusions. They concluded that IgE-mediated allergic reactions played a role in the pathogenesis of otitis media with effusion in about 23% of young allergic patients with this disease. They proposed that the portion of the upper respiratory tract involved in the IgE-mediated hypersensitivity reaction was the mucosa of the nasopharyngeal portion of the eustachian tube, which becomes inflamed. This study found evidence of allergy in 80% of CSOM compared with 47% of ASOM and 33% of controls. This finding was similar to that of Skoner. (2004). In their study of 209 children with OME they found allergic rhinitis in 89%, asthma in 36%, and eczema in 24%. This high prevalence of allergy led to suggestion of a causal relationship.

The finding of significant correlation between total MES IgE level in acute and chronic otitis media (P= 0.04) appeared to further support a likely role for IgE level as

a potentiating factor of chronicity. However, secondary bacterial and viral infection might contribute to the persistence of otitis media. Recurrent suppurative otitis media (SOM) has been reported to occur in about one-third of the allergic rhinitis population. The increasing prevalence of otitis media has been linked to nasal allergy (Mogi and Suzuki, 1997; The Allergy Report, 2000; Umapathy *et al*., 2007). The role of the middle ear in allergy and the origin of Ig E are still controversial. Clinical and experimental studies showed the efficacy of allergic treatment in improvement of otitis media in patients with both SOM and allergy (Bernstein, 1992; Mogi *et al.,* 1992; Lanphear, 1997). Further, viral-specific IgE antibodies similar to those of type I allergy has been found to be induced by upper airway infections (Bernstein and Doyle, 1994; Mogi and Suzuki, 1997; Hurst *et al.,* 1999).

Mogi and Suzuki. (1997) reported otitis media in 42% of nasal allergy and 35% of non allergy children, a ratio which was significantly higher than those seen in the control group. They also showed that the eustachian tube was involved in type I allergic reactions of the nose. The tubal dysfunction elicited was transient, although, it interfered with the clearance of MES. They concluded that type I allergic reactions of upper respiratory tracts were factors indicative of a chronic state of disease, rather than a cause of SOM. Pathophysiologic models of otitis media have shown eustachian tube obstruction by both intrinsic venous engorgement and extrinsic mucus plugs secondary to IgE-mediated hypersensitivity (Watanabe *et al.,* 1991; Mogi *et al.,* 1992; Alles *et al.,* 2001; Chantzi *et al.,* 2006). This resulted in significant reduction of middle ear pressure which disrupted tight junctions and allowed for transudation of fluids into the middle ear space. Hence further middle ear inflammation, mucosal metaplasia, and increased glandular activities, all of which were hallmarks of chronic otitis media with effusion (Bernstein, 1992; Bernstein and Doyle, 1994; Hurst *et al.,* 1999; Chantzi *et*

al., 2006). Further, experimental studies in guinea pigs have shown that mast cells were distributed in the tubotympanum in response to continuous inflammatory stimuli. Their densities were highest in the pharyngeal orifice of the eustachian tube and this density was higher in the adult than in developing guinea pigs (Mogi and Suzuki, 1997; Sobol *et al.,* 2002; Chantzi *et al.,* 2006). The induction of viral-specific IgE by viral infections of the upper respiratory tract and the triggering of bacterial infection by viruses has been reported (Watanabe *et al.,* 1991; Hurst *et al.,* 1999; Alles *et al.,* 2001; Skoner, 2004). In this study we assayed the total Ig E and not the specific IgE due to the limitations in our facility, similarly, total IgE was used in the reports of others (Bernstein, 1992; Mogi and Suzuki, 1997; The Allergy Report, 2000). The associated parasitic infection was ruled out by checking the blood of the patients for eosinophilia which was not found, although we did not check the stool for parasites. The age of the patients ranged from 6 months to 9 years while the age inclusion criterion was 0 – 12 years. This discrepancy was due to the absence of patients at the extremes of the inclusion criteria available for enrollment. However, we noted that the range of the total IgE assay might also be due to the wide range of the age of the subjects as a 9 year old was far more likely to exhibit an allergic constitution than a 6 month old.

From the finding of significant history of allergy and positive skin reaction in 70% of CSOM compared to 28% in resolved ASOM, it was concluded that there might be substantial potentiating role of allergy in SOM. Other factors such as malnutrition and overcrowding might also encourage viral and bacterial infection leading to persistence of suppurative disease.

5.7. Interferon Gamma in Suppurative Otitis Media

The main finding from this study was the significant association between elevated middle ear secretion of IFN-γ and the tendency to chronicity, in addition there was higher secretion of IFN-γ in mucoid OM compared to purulent OM.

This is similar to the work of Chen *et al.* (2005) and others (Himi *et al.,* 1992; Yellon *et al.,* 1995; Maxwell *et al.,* 1997). Chen *et al.* (2005) reported that the level of IFN-gamma in chronic period was much higher than in acute period. They concluded that high expression of IFN-gamma in the middle ear effusion may be a reference of SOM tending to chronic course and that the IFN-gamma in the middle ear effusion may be produced by local tympanum, but not exuded simply from blood.

Similarly, Yellon *et al.* (1995) and Himi *et al.* (1992) found that higher level of IFN secretion in middle ear effusions may be a marker for OM chronicity. Maxwell *et al.* (1997) found that the TNF-alpha levels were significantly lower for children with multiple tube insertions (P = 0.02). Higher levels of TNF-alpha were noted in those children who subsequently developed episodes of otitis media after tube placement (P = 0.02), hence they concluded that cytokines and their inhibitors are present in a large number of middle ear effusions and in part are likely important in the regulation of inflammatory processes in chronic otitis media with effusion.

IFN γ is produced by the T – lymphocytes in response to viral or bacterial challenge and it has been shown to activate macrophages, stimulate cytotoxic cell activity and increase B – cell differentiation and antibody production, and, it has potential to cause tissue damage (Yellon *et al.,* 1991; Nassif *et al.,* 1997). The cytotoxic and osteoclast and fibroblast stimulating activity may be responsible for the persistence of otorrhoea seen in the patients (Yellon *et al.,* 1991; Himi *et al.,* 1992; Maxwell *et al.,* 1997; Nassif

et al., 1997). In an earlier study, assessing immunoglobulins in suppurative OM, serum IgG level (P= 0.04), MES Ig G (P= 0.02) and MES IgE (p = 0.04) showed significant difference between ASOM and CSOM suggesting their role as a chronicity factor (Hotomi *et al.,* 1994; Maxwell *et al.,* 1997; Lasisi *et al.,* 2008).

In this study, correlation was found between IFN-γ and MES immunoglobulins, suggesting that the MES IFN-γ was secreted from the middle ear mucosa. In addition, correlation was also reported between MES IFN-γ and other cytokines and immunoglobulins in the middle ear (Yellon *et al.,* 1991; Himi *et al.,* 1992; Maxwell *et al.,* 1997; Nassif *et al.,* 1997). The explanation for the reverse correlation was that a high MES IFN-γ resulted in mucosal damage leading to reduced secretion of protective immunoglobulins in the middle ear thus leading to chronicity.

Similarly, IFN-γ helps in the production of TNFa, which has been implicated in the increased secretion of mucin by airway epithelium, hence persistence of otorrhoea. Induction of mucin secretion has been reported to be via an intracellular pathway that appears to involve endogenously produced nitric oxide (Fitzgerald *et al.,* 1987; Yellon *et al.,* 1991; Hotomi, 1994; Nassif *et al.,* 1997; Storgaard *et al.,* 1997; Lasisi *et al.,* 2008b). A potential pathway by which cGMP could enhance mucin secretion involves activation of phosphokinase G which are serine kinases involved in a such biologic responses as calcium signaling, neutrophil degranulation, potassium and chloride channel functioning, and mucin secretion (Yellon *et al.,* 1991; Hotomi, 1994; Nassif *et al.,* 1997; Storgaard *et al.,* 1997). A major significance of our findings is that chronicity might be associated with high MES IFN-γ, however, this study did not show causal relationship between high IFN-γ and ASOM. The clinical implication of this study is that by assessing the Interferon Gamma level in the ME aspirate, the parents

can be better informed that the ear infection may linger on for longer period, and that the child may need longer term follow up. It is scientifically probable that control of chronicity of otitis media could be achieved by the trial of agents which can counteract the effects of IFN-γ, among other Th 1 cytokines. However, the study did not look into the role of the size and bacterial load of adenoids in relation to the outcome of suppurative otitis media. It was concluded from this study that high MES IFN-γ is a chronicity factor in suppurative OM.

5.8. The Role of Nutritional Factors in the Aetiology and Outcome of Suppurative Otitis Media

The main finding in this study was significant hyporetinolaemia in ASOM subjects compared to controls (P<0.05), suggesting an association between the deficiency of the role of retinol and development of ASOM. This was similar to the findings of Yilmaz *et al.* (2004) and Cemek *et al.* (2005). Another major contribution of this study, which has not been reported earlier, was the finding of significantly low serum retinol in ASOM patients who progressed to chronicity compared to those who showed evidence of healing. This suggested a possible role of hyporetinolaemia in the potentiation of chronicity of suppurative otitis media. This could be explained by the role of Vitamin A in maintaining the integrity of epithelia and secretion of mucus, which may be defective in patients with low serum level. Retinoid and their derivatives are required for the maintenance of the normal epithelial mucociliary phenotype. They exert their effects via specific nuclear receptors, retinoic acid receptors and retinoid X receptors all of which are members of the steroid/thyroid receptor super-family. They act as ligand-dependent transcription factors, interacting with retinoic acid response elements located in promoter regions of target gene (Andersson *et al.,* 1986; WHO, 1995).

Reverse transcriptase/polymerase chain reaction and immunohistochemistry have been used to detect all these receptors in vitro and in vivo in normal human lung tissue, oral tissue and bronchial specimens (Aukrust *et al.,* 2000; Baeten *et al.,* 2002; Baeten *et al.,* 2004). On the other hand, in this study, serum zinc was not found to be a significant factor in development of ASOM and predisposition to chronicity.

Zinc is an essential trace element important for immune function and resistance to infection. It must be consumed regularly as it cannot be stored in the body. Over 60% of children under the age of five have zinc deficiency from inadequate diets in some low income countries (Caulifield *et al.,* 2004). While vitamin A deficiency was found in an estimated 127 million preschool children and 7.2 million pregnant women worldwide, suggesting a serious public health problem (West, 2002). The two nutrients play essential role in immune function and their effects included increased risks of mortality and morbidity from measles, respiratory tract infections, diarrhea diseases (Kirby *et al.,* 1981), blindness (Semba and Bloem, 2002), and anemia among others (Kirby *et al.,* 1981; Christian *et al.,* 2000; Semba and Bloem, 2002). In addition, their supplementations have been reported to reduce morbidity rates associated with pneumococcal disease by delaying the rate of colonization and the age of occurrence (Christian *et al.,* 2000). Retinol and Zinc were chosen because they are nutritional factors which also have antioxidant properties and the role of oxidative stress has been emphasized in otitis media (Craft *et al.,* 2000).

In a study of 23 patients, Cemek *et al.* (2005) reported a significantly decreased serum level of retinol in the patients with acute otitis media ($P<0.05$). The deficiency of the immunomodulatory functions associated with retinol has been reported as the critical factor in the predisposition to infections. Vitamin A deficiency induces an up-

regulation of the T helper subset 1 cell (Th1)–mediated response (Bhandari *et al.,* 1994; DeCicco *et al.,* 2001). Experiments in vitro and animal studies suggested that retinoid were important regulators of monocytic differentiation and functions. When added to monocytic, myelomonocytic or dendritic cell line cultures, retinoic acid promotes cellular differentiation (Breitman *et al.,* 1980; Geissmann, 2003; Jiang *et al.,* 2003; Mohty *et al.,* 2003). In addition, it influences the secretion of key cytokines produced by macrophages, including tumor necrosis factor (TNF-α), IL-1ß, IL-6, and IL-12. All-trans-retinoic acid skewed the differentiation of human peripheral blood monocytes to IL-12-secreting dendritic cells in one in vitro study (Jiang *et al.,* 2003;, Mohty *et al*., 2003; Lasisi, 2009). In another study, it inhibited lipopolysaccharide-induced IL-12 production by mouse macrophages (Na *et al.,* 1999). All-trans-retinoic acid was shown to decrease secretion of TNF-α in murine peripheral blood mononuclear cells (Kim *et al.,* 2004; Mou *et al.,* 2004) and myelomonocytes (Mathew and Sharma, 2000) and macrophages (Hashimoto *et al.,* 1998). On the other hand, retinoid appear to enhance the secretion of IL-1ß (Hashimoto *et al.,* 1998; Higuchi and Nagata, 2000; Abass and Lichtman, 2003; Cherian *et al.,* 2003; Ribeiro *et al.,* 2003; Maun *et al.,* 2004) and IL-6 (Ribeiro *et al.,* 2003) by macrophages and monocytes.

The development of neutrophil in the bone marrow is controlled by retinoic acid receptor-modulated genes (Maun *et al.,* 2004), and retinoic acid in cultures accelerates neutrophil maturation (Ribeiro *et al.,* 2003). Treatment with retinoic acid has been shown to restore the number of neutrophil and the superoxide - generating capacity in rats and calves (Higuchi and Nagata, 2000; Cherian *et al.,* 2003).

There are limited data on the relationship between vitamin A and neutrophil function in humans. Rahman *et al.* (1996) examined the effect of vitamin A supplementation on

cell-mediated immunity among infants younger than 6 months in Bangladesh. Their results showed cell-mediated immunity responses were improved among infants with adequate serum retinol concentrations after supplementation, but there was no improvement among children with low serum retinol levels.

The impact of vitamin A on circulating effectors of innate immunity, including acute-phase response proteins and the complement system, was studied in trials from Ghana, Indonesia, and South Africa. In the Ghana study of preschool children, large doses of vitamin A every 4 months for 1 year resulted in significantly increased serum amyloid A and C-reactive protein among children (Fawzi *et al.,* 1993). However, no effect was found on the C-reactive protein concentrations in the Indonesia study (Semba *et al.,* 2000). Plasma C3 complement was not affected by four doses of vitamin A administered within a 42-day period to South African children (Beisel, 1982; Zhao and Ross, 1995).

Another significant risk factor in the pathogenesis of acute otitis media which has also been found to be adversely affected by low retinol is nasopharyngeal colonization. Cole *et al.* (2001) reported that the risk of nasopharyngeal colonization among infants aged 4 months who were not colonized by age 2 months was significantly reduced in the vitamin A group compared with the placebo group [odds ratio 0.51 (0.28, 0.92), P = 0.02). The odds of colonization were 27% lower in the vitamin A treated group than in the placebo group [odds ratio 0.73 (0.48, 1.1), P = 0.13]. The risk of colonization with penicillin-resistant isolates was 74% lower in the vitamin A treated group than in the placebo group at 2 months of age. Hence they concluded that neonatal vitamin A may play a role in lowering morbidity rates associated with pneumococcal disease by delaying the age at which colonization occurs.

Physicochemical injuries alter the respiratory epithelium resulting in a multistep process to squamous metaplasia (Beaton *et al.,* 1993; Benn *et al.,* 1997; Bahl *et al.,* 2002). In vivo, vitamin A deficiency induces replacement of the normal pseudostratified mucociliary epithelium by a metaplastic stratified squamous epithelium (Benn *et al.,* 2002; Benn *et al.,* 2003), a process that can be reversed by a dietary vitamin A supplement (Benn *et al.,* 1995; Benn *et al.,* 2000). Retinoic acid (RA) inhibits the expression of squamous-related genes, such as keratin 13 in rabbit tracheobronchial cells (Bhaskaram and Rao, 1997), cholesterol sulfate in human epithelial cells (Binka *et al,* 1995), and transglutaminase and cornifin in human epidermal keratinocytes (Breitman *et al.,* 1980). RA also induces a mucosecretory phenotype by activating both transcription of specific mucin genes, particularly MUC2, MUC5B and MUC5AC, and mucin secretion (Moon *et al.,* 2000; Hwang *et al.,* 2006). All of these result in alteration of mucin secretion which could be a factor in persistence of otorrhoea and CSOM.

Inferring from these findings, supplementation of vitamin A in children might help in control of ASOM and its chronicity. Furthermore, this has been corroborated by findings from vitamin A supplementation studies (Hussey and Klein, 1990; Coutsoudis *et al.,* 1991; Brown and Robert, 2004). Studies from England, South Africa and Tanzania have reported decrease in the risk of nutritional blindness and morbidity of infectious origin from measles, respiratory infections, severe diarrhea, HIV, and intestinal helminthiasis following periodic vitamin A supplementation to children (Hussey and Klein, 1990; Coutsoudis e*t al.,* 1991; Brown and Robert, 2004).

The effects of vitamin A supplementation on child morbidity include a reduction in the severity of measles. This could be correlated with the enhanced T-cell-dependent

antibody production that was observed in the children with measles (Fawzi e*t al.,* 1993; Fawzi *et al.,* 2000). A decrease in the severity of measles morbidity could also explain an overall average reduction in measles-specific mortality of about 60% (Grotto *et al.,* 2003). This could have a positive effect in the control of suppurative otitis media. The benefits of vitamin A on measles-related outcomes may go beyond the correction of underlying deficiencies and could actually represent adjuvant therapeutic effects (Butler *et al.,* 1993). In addition, the degree of depression of retinol was associated with severity of these illnesses. The beneficial effects of vitamin A supplementation among children could be mediated by a short-term increase in antibody production, possibly as a result of increased lymphocyte proliferation. However, more data are needed from human trials about the role of vitamin A supplementation in modulating the Th1/Th2 response as a potential explanatory mechanism for the vitamin's observed effects on clinical outcomes.

There was only one report, that of Bondestam *et al*. (1985), linking the recurrent otitis media with low serum Zinc. Although, several reports by Walker *et al.* (2004) and others (Juni *et al.,* 2001; Bhutta, 2004; Robbersdad *et al.,* 2004; Higgins and Green, 2005) have shown the benefits of zinc supplementation on prevention of otitis media. In this work, we have found that serum zinc was a factor in the type of middle ear secretion. Lower serum zinc was found in the MOM than the POM. Allergy played significant role in MOM through the secretion of histamine and other mediators. In contrast, zinc, Vitamin C and bioflavonoid have been reported to help in reducing the effects of histamines that are secreted by the body during an allergic episode (Bondestam *et al.,* 1985). Thus it is plausible that the relative absence of zinc may explain the link.

In conclusion, this study had identified hyporetinolaemia as a significant factor in the aetiology and outcome of suppurative otitis media, suggesting retinol supplementation as one strategy in control of SOM. However, there is need for a follow - up of this study to test the effect of vitamin A supplementation on the outcome of AOM

5.9. The Future of Research in Immunobiology of Suppurative Otitis Media

5.9.1. Epidemiology

Most studies have looked into the epidemiology, documenting the incidence up to 90% in children under 2 years of age (Juhn et al., 1994; Casselbrant et al., 1995) and 60% in preschool attendees (Casselbrant et al., 1995). In Nigeria, the prevalence is 21.2% in children and accounted for 25% of patient attendance at the otorhinolaryngologic clinic (Paradise et al., 1997; Amusa et al., 2005). The Sociodemographic risk factors have been reported with differences in the significance and roles of the risk factors. Most of the works were point survey and review of community/ hospital data, but very few were prospective longitudinal study of children.

This study followed up children for one year from birth and found the prevalence of suppurative otitis media in the first year of life to be 37%. In addition the study found that low levels of cord blood IFN gamma, retinol and Zinc were significant in development of ESOM. The hypothesis was that the mechanism of prenatal influence might be expressed through maternal undernutrition particularly in the trace elements and overwhelming viraemia passed down to the infant at birth and predisposing to OM. Serial measurement of fetal immunbiologic markers and the maternal serum level of these markers might also be important in future to establish relationship between maternal and fetal undernutrition and early otitis media. Indeed studies on prenatal predisposition to OM are few and these are areas for further studies in the immunobiology of the development of OM. In addition, the study did not consider the

effect of breastfeeding, although, all the children were on breastfeeding during the study. Chantzi *et al.* (2006) concluded that infants who were fully breastfed for 4 to < 6 months had statistically significant increased risk for both pneumonia (odds ratio [OR]: 4.27; 95% confidence interval [CI]: 1.27-14.35) and > or = 3 episodes of OM (OR: 1.95; 95% CI: 1.06-3.59) in those who were fully breastfed for 4 to < 6 months compared with > or = 6 months. Hence this supports the current recommendations that infants should receive only breast milk for the first 6 months of life.

One other significant finding from this study was that the otorrhoea in the children were either pus or mucoid, the study did not encounter the typical serous effusion as reported in cauccasians, despite the fact that children in the first year of life were studied. This further reinforced earlier observation in the out – patient clinic. Earlier investigators have posited that otitis media with effusion was not commonly seen in this environment the patients presented late and there was secondary bacterial infection (Casselbrant *et al.,* 1995; Paradise *et al.,* 1997; Smirnova *et al.,* 2004; Lasisi *et al.,* 2008). However, the present study was a prospective follow up design, thus obviating the problem of late presentation. This difference in the nature of otorrhoea need further study to be clarified. This might also suggest the need to study the role of the eustachian tube factor in the current cohort of patients. Probably, these findings may change the current knowledge of the pathogenesis of OM, hence the clinical management.

5.9.2. Immunoglobulins and OM

The activity of immunoglobulins in chronic OME is evidence of chronic humoral inflammatory processes in the middle ear, which is obviously controlled by cytokines. The presence of the main types of immunoglobulins, IgM, IgG, IgA, secretory IgA and IgE, in effusions is indirect evidence that cytokines IL-2, IL-10, TGF-b, IL- 4 and IL-

5, which are involved in regulation of immunoglobulin production and secretion also regulate humoral immune reactions during the course of middle ear inflammation (Jelinek and lipsky, 1987; Florentino *et al*., 1991; Defrance *et al.,* 1992; Rouset *et al.,* 1992; Brown and Hural, 1997; Salvi and Holgate, 1999). Different types of immunoglobulins, namely IgM, IgG, IgA, secretory IgA and IgE, have been identified in effusions and middle ear fluid of chronic OME (Liu *et al.,* 1975; Lang *et al.,* 1976; Lewis *et al.,* 1978; Jones *et al.,* 1979). The immunologic investigation of effusions detected the immune complexes of IgG (IgG-/ICs) and IgA (IgA-/ICs) in both acute and chronic otitis media (Tamanaka *et al.,* 1985; Yamanka *et al.,* 1987). The highest level of IgG-ICs was found in sub-acute cases, whereas IgA-ICs were predominant in chronic OME. The immunoglobulin immune complexes have been speculated to prolong the inflammatory process in the middle ear. The presence of immunoglobulins in chronic OME was associated mainly with the bacterial infection (Lewis *et al.,* 1979; Stenfors and Rasanen, 1991a; Stenfors and Raisanen, 1991b; Takada *et al.,* 1998). IgG and IgA antibodies specific to Hemophilus influenzae and S. pneumoniae, (Lewis *et al.,* 1979) IgG, IgM, IgA and secretory IgA antibodies specific to outer membrane antigens of Moraxella catarrhalis (Takada *et al*, 1998), and *Staphylococcus aureus*-harboured bacteria, intensely coated with secretory IgA and IgG antibodies were identified in chronic effusions (Stenfors and Rasanen, 1991a; Stenfors and Raisanen, 1991b). However, only the secretory immunoglobulin, IgA, was identified in effusions infected with respiratory viruses (Yamaguchi *et al.,* 1984). High levels of MES IgE and IFN gamma, and impaired middle ear immune response as shown by low levels of MES IgA and serum Ig G were identified as factors of chronicity in OM (Lasisi *et al* 2008a, Lasisi *et al* 2008b). The nature of otorrhoea has also been linked to the differences in the concentration of the immunoglobulins in the middle ear secretion.

The mucoid type of effusions contained a high level of IgG, IgA (Liu *et al.,* 1975) and IgE (Lim *et al.,* 1985). However, these findings showed higher Ig A in mucoid and lower IgG and IgE in mucoid compared to purulent effusion. Correlation between the levels and types of immunoglobulins and the immunoregulatory and allergy-associated cytokines in chronic OME has not been investigated. Comparison of the immunoglobulin levels measured in effusions and in sera showed that, in many cases, the effusion level of secretory IgA (Jones *et al.,* 1979) and IgE (Bernstein *et al.,* 1985) was significantly higher than the corresponding serum level, and this was hypothesized to be the evidence of local overproduction of immunoglobulins in the middle ear. It is still not known whether the immunoglobulin in the middle ear secretion is produced from the middle ear or from the nasopharynx and subsequently transported into the middle ear. This may require animal experiment to be confirmed. In addition, differences between the cellular constituent of the secretion and the mucosa may reveal the types of cells produced by the middle ear. Further investigative studies are required in this area.

5.9.3. Cytokines in OM

Cytokines are responsible for resolution of inflammation and can initiate local molecular processes leading to histopathological changes in the middle ear mucosa and submucosa, and the chronic condition of otitis media (Juhn *et al.,* 1994; Smirnova *et al.,* 2004). Different groups of cytokines have been identified in the middle ear effusions and mucosa: The pro-inflammatory TNF-a, IL-1b, IL-6 and IL-8; the immunoregulatory IL-2, IL-10 and TGF-b; and the allergy-associated IL-4, IL-5 and GM-CSF (Yamanaka *et al.,* 1987; Defrance *et al.,* 1992; Rouset *et al.,* 1992; Juhn *et al.,* 1994; Smirnova *et al.,* 200).

The immunoregulatory cytokines and the allergy-associated cytokines have been considered the key regulators of the middle ear inflammation responsible for the molecular and cellular background of chronic OME (Smirnova *et al.,* 2004). The immunoregulatory cytokines IL-2, IL-10 and TGF-b initiated and supported molecular switching of the acute phase of inflammation in the chronic stage (Smirnova *et al*., 2004). Whereas the allergy-associated cytokines IL-4, IL-5 and GM-CSF very probably provided the molecular and cellular background for chronic humoral, cell-mediated and allergic inflammatory processes in the middle ear, which lead to the chronicity of OM (Smirnova *et al.,* 2004). However, further studies are necessary in order to elucidate the molecular mechanisms of cytokine regulation of the middle ear inflammation; and the possibility of anti-cytokine therapy in clinical treatment of OM. Animal models suggested that the inflammatory stimulus were bacterial endotoxin, which would stimulate TNFa production, and led to mucin production and mucous hyperplasia (Ball *et al.,* 1997; Demaria *et al.,* 1997; Rose *et al.,* 1997).

Yellon *et al.* (1991) detected interleukin-1 beta in 58% (44/75) middle ear effusions; interleukin-6, 83% (60/72); tumor necrosis factor alpha, 37% (28/75); and interferon gamma, 61% (45/74). High TNFa levels in middle ear effusions have been correlated with persistence of OME. One function of IL-8, in the middle ear as elsewhere, is to induce chemoattraction of neutrophil and its production is directly controlled by TNFa and IL-1b. IL-8 has been found in higher levels in more viscous effusions and in effusions with bacteria on culture. TNFa and IL-1b are markers of the acute inflammatory response, whereas IL-8 +may represent chronicity (Yellon *et al*., 1991; Nassif *et al.,* 1997; Hotomi *et al.,* 1994; Storgaard *et al.,* 1997; Fitzgerald *et al.,* 1987; Fitzgerald *et al.,* 1988; Florentino *et al.,* 1991a; Rousset *et al.,* 1992a; D'Andrea *et al.,* 1993; Ho and More, 1994).

IL-2 is the up-regulating cytokine, which stimulates primarily the cell-mediated inflammatory response by promoting growth, proliferation and differentiation of T cells, B cells, natural killer (NK) cells, monocytes and macrophages. It is secreted mainly by activated T cells. IL-2 induces cytokine production in T cells, including interferon (IFN)-g and IL-4 (Trinchieri *et al.,* 1984; Malkovsky *et al.,* 1987; Baccarini *et al.,* 1989; Florentino *et al.,* 1991b; Wang *et al.,* 1994).

In contrast, IL-10 (known as the cytokine synthesis inhibitory and macrophage deactivating factor) down-regulates the immune reactions accompanying acute inflammation and limits the duration of inflammatory responses. IL-10 can also promote and regulate chronic inflammatory processes. IL-10 is produced by a variety of cell types, including CD4 - T cells, activated CD8- T cells and activated B cells. The main anti-inflammatory activities of IL-10/namely, inhibition of cytokine production in macrophages, neutrophil, T cells and NK cells (Florentino *et al.,* 1991b; Wang *et al.,* 1994), and inhibition of the macrophage -/monocyte activation and the antigen presentation abilities of these cells lead to the resolution of inflammation. However, if the acute inflammatory process has not been resolved, IL-10 can induce humoral inflammatory reactions such as the immunoglobulin isotype switching in B cells (Defrance *et al.,* 1992; Rousset *et al.,* 1992) and differentiation of B cells into plasma cells (Rousset *et al.,* 1995) and thus promote switching of inflammation in the chronic stage. Identification of factors involved in chronicity appears to be an essential step in the treatment and ultimate prevention of chronic otitis media. Our finding revealed significant difference in the middle ear secretion levels of interferon gamma between resolved ASOM and CSOM, and between mucoid and purulent OM. This suggested that the middle ear immune response may be a significant factor in the outcome of SOM. Further hypothesis is that persistence of SOM may be a result of

deficient secretion of the pro – inflammatory cytokine compared to the immunoregulatory cytokines. The study of the ratio of these cytokines in SOM should constitute major areas of future research because of their clinical implications in the control of CSOM.

5.9.4. Allergy and OM

Allergy has been associated with OME in 35% to 45% of cases (Barenkamp, 1986; Derebery and Berliner, 1997; Homoe *et al.,* 1999). In addition, sensitivity to foods and inhalants has been reported in 92.3% and 100% respectively of patients with eustachian tube dysfunction (Mandel *et al.,* 2008; Rovers *et al.,* 2008). Middle ear effusions contain mediators of the allergic response, such as IgE and eosinophil cationic protein children (Chole 1979, Manning and Wright, 1992; Semba *et al.,* 2000; Aladag *et al.,* 2007). However, from the relative concentrations of the mediators in the effusion and serum it is yet to be confirmed that they are produced locally (Chole, 1979; Yilmaz *et al.,* 2004; Cemek *et al.,* 2005; Aladag *et al.,* 2007). In addition, the presence of IgE in chronic OME has not been associated with local allergic inflammation (Lim *et al.,* 1976; Sloyer *et al.,* 1980). However, local overproduction of IgE was usually accompanied by local allergic reactions, such as degranulation of mast cells found in the middle ear biopsy specimens (Lim *et al.,* 1976) and expression of IgE on mast cells detected in nasal mucosa specimens from patients with OME (Sloyer *et al.,* 1980). In a study of 228 acute SOM subjects, we identified positive skin test to one or more of the allergens among dust, house dust mite, mould, cockroach and poultry feather in 105 compared to 15/71 controls. Allergy was found in 66/87 of CSOM and 39/141 of resolved ASOM. In addition, there was significant difference in the mean middle ear secretion levels of Ig E between resolved ASOM and CSOM. All of these revealed that allergy was a significant factor in the persistence of SOM.

However, it was difficult to prove whether IgE was produced in the middle ear or a result of migration into the middle ear space. In addition we did not study allergy associated cytokines in our study due to limited research funds.

Allergy associated cytokines (IL-4, IL-5, granulocyte macrophage colony-stimulating factor), as crucial molecular regulators, responsible for chronic inflammation in the middle ear and the chronic condition of OME (Smirnova, 2004). IL-4 was identified in the middle ear effusions of children with persistent OME (Jang and Kim, 2002) and in atopic children with OME undergoing myringotomy and ventilation tube placement (Sobol *et al.,* 2002). The analysis of effusions showed a higher mean level of IL-4 in the allergy-positive group compared with the allergy negative group (Jang and Kim, 2002) and a higher percentage of cells expressing IL-4 in atopic patients with OME compared with that seen in non-atopic patients (Sobol *et al.,* 2002). A higher level of IL-4 in effusions correlated with predominance of T lymphocytes, which was the sign of chronic inflammation and was also related to the atopic background of patients with OME (Sobol *et al.,* 2002). It was thus speculated that the levels of the allergy associated cytokines may correlate with the middle ear IgE; and persistence of SOM. These are areas for future research in addition to the role of IL 4 and IL 5 in the aetiology and potentitiation of OM.

5.9.5. Mucin Factor in OM

The chronic condition of otitis media is associated with proliferative changes in the middle ear tissues, especially in the surface middle ear mucosa, which is present in OM as a modified pseudostratified epithelium (Moller and Dalen, 1979; Meyerhoff and Giebink, 1982). In addition, goblet cells are proliferating with enhanced secretory activity (Tanaka *et al.,* 1986) and formation of mucus glands occurs (Goycoolea, 2001; Tos and Caye-Thomasen, 2002). Goblet cells produce and secrete mucin, which are

important glycoprotein in the mucociliary transport system of the middle ear and are the main component of middle ear effusions, responsible for the viscous properties of effusions (Fitzgerald *et al.,* 1989; Carie *et al.,* 1992). However, under disease conditions, alterations that occur in the middle ear and eustachian tube mucin metabolism (Lin *et al.,* 2001), in the structure of mucin glycoproteins (Hutton *et al.,* 1993; Hutton *et al.,* 1998) and in the glycoconjugate expression in cilia and goblet cells (Sone *et al.,* 1998; Takeuchi *et al.,* 2003) promote the dysfunction of the normal mucociliary transport system and the formation of effusion in the middle ear cleft. The expression of several mucins has been detected in OME (Hutton *et al.,* 1993; Hutton *et al.,* 1998). However, overproduction of only two mucin types has been observed in otitis media; namely, the membrane-bound MUC4 (Lin *et al.,* 2001) and the secreted MUC5B (Hutton *et al.,* 1998; Sone *et al.,* 1998; Lin *et al.,* 2001; Takeuchi *et al.,* 2003). Another secreted mucin (MUC5AC) is always present in effusions, but in varying amounts (Hutton *et al* 1998, Kawano *et al* 2000, Lin *et al.,* 2001; Smirnov*a et al.,* 2002), and its levels might be linked to the levels of the pro-inflammatory cytokines TNF-a, IL-6 and IL-8, which could promote different levels of MUC5AC secretion (Smirnova *et al.,* 2001; Smirnova *et al.,* 2002). This study showed that hyporetinolaemia was significant in the development and potentiation of otitis media (Lasisi, 2009). Earlier investigators have linked low retinoic acid with changes in the viscosity of the mucin in favour of development of OM. Further role of retinol is in augmenting epithelial integrity, thus ensuring optimal epithelial recovery following inflammation. Thus we speculate that delayed epithelial recovery may be one of the ways in which hyporetinolaemia leads to persistence of SOM. However, further proof in support of this may require an animal experiment and an interventional study in SOM subjects. Interventional studies from India and other developing countries have

reported improvement in the resolution of other RTI although this has not been done with otitis media, hence further studies are required in this area.

5.9.6. Biofilm Basis of Chronicity

The demonstration that otitis media, as a chronic infectious disease, is a human pathologic condition associated with biofilm development represent a major advance in the understanding of SOM. The concept that biofilms are the primary source of bacterial infection in chronic forms of otitis media is novel (Costerton *et al.,* 1999; Stoodley *et al.,* 2002; Costerton *et al*., 2003; Hall - Stoodley *et al.,* 2004).

It has long been recognized that most bacteria in nature exist in a biofilm state as opposed to the small percentage of bacteria that live in a planktonic or free-swimming state. In the planktonic state, bacteria are capable of producing host responses that are generally associated with acute infections: fever, inflammation, cellular damage and leukocyte stimulation. However, pathologic conditions in which bacteria form biofilms also have numerous clinical implications. In the biofilm state, bacteria become organized, anchor to a surface and form a microcolony surrounded by a complex polymeric matrix that is constantly being remodeled, which includes polysaccharides, nucleic acids and proteins that are secreted by the bacteria themselves. Bacteria in the biofilm state maintain a low metabolic rate, are able to escape host immune surveillance and rapidly share genetic information. These characteristics make biofilm bacteria difficult to culture using standard techniques, difficult to eradicate with antimicrobial compounds and allows for the development of effective and widespread antimicrobial resistance characteristics.

Evidence that chronic otitis media is the result of a biofilm infection has been demonstrated by a number of well designed studies (Rayner *et al.,* 1998; Ehrlich *et al.,* 2002). However, recent identification of bacterial biofilms in children with recurrent

acute otitis media and chronic otitis media with effusion (OME), and not in control populations has brought this otitis media biofilm discussion from a basic science, theoretical discussion to a clinical one (Hall – Stoodley *et al.,* 2006).

With the demonstration of biofilms in children with chronic otitis media, and the likelihood that these biofilms play a role in the pathogenesis of otitis media, important clinical questions require additional scrutiny. What are the implications for otitis media treatment and pathogen eradication with the knowledge that middle ear pathogens routinely achieve biofilm formation and that this environment enhances their ability to develop and acquire antimicrobial resistance? Early onset of otitis media increases the likelihood of developing chronic disease. What are the implications of biofilm formation in these patients and the best strategies for prevention? What will be the clinical impact of antibiofilm interventions? As middle ear biofilms are likely to originate in the nasopharynx, this may include strategies such as reduction of nasopharyngeal biofilm through surgical (adenoidectomy), mechanical or pharmacologic (nasal irrigations or medications designed to reduce biofilm formation) means. It has been postulated that each of these recurrent infections represent a new episode caused by a unique bacterial strain ascending through the nasopharynx and eustachian tube into the middle ear space (Pettigrew *et al.,* 2004). However, these findings would suggest that viable bacteria are capable of residing as biofilms within the middle ear space in between acute exacerbations of bacterial infection and, at least in part, may be responsible for the recrudescence of these acute infections through planktonic showering of bacteria from the biofilm.

Other studies have supported this hypothesis and have demonstrated that approximately 30% of cases of recurrent otitis media result from relapses attributable to the original organism (Harabuchi *et al.,* 1994). In addition, surgical intervention

with tympanostomy tubes for patients with recurrent OM and OME, known to be clinically efficacious, will need further examination with a biofilm concept of pathogenesis. This includes not only initial placement of tympanostomy tubes but also potential complications such as chronic otorrhea from tympanostomy tubes (Malaty *et al.,* 2008).

In conclusion, the biofilm concept did not exclude other potential pathogenic factors associated with chronic otitis media. Important associations such as an antecedent viral upper respiratory infection, eustachian tube dysfunction, allergy, a genetically predisposed host, persistent inflammatory mediators or exacerbation by gastroesophageal reflux could each be incorporated into the concept of facilitating biofilm formation for further studies. These findings might provide significant scientific advances toward understanding chronic otitis media and developing novel treatment regimens.

This study employed the ELISA techniques and high performance liquid chromatography; however more sophisticated methods such as the Polymerase Chain reaction (PCR) and electron microscopy are commonly available in the developed world. The PCR technique has been used to identify microorganisms and their product while electron microscopy technique is used in visualizing the biofilm. It is suggested that these new technologies be employed in the investigation of middle ear secretions in the OM for microorganisms and their products

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Otitis media has a complex multifactorial pathogenesis which can be provoked by different primary factors such as bacterial and viral infections, local allergic reactions and reflux. The typical inflammatory response being the accumulation of cellular and chemical mediators in middle ear (Smirnova *et al.,* 2004). However, there are controversies whether specific biochemical and immunochemical factors might be responsible for the aetiology or chronicity of otitis media (Juhn *et al.,* 1994).

The incidence per live birth of early suppurative otitis media was 37%. The significant factors included sociodemographic variables such as malnutrition, indoor cooking and allergy. In addition, decreased concentrations of cord blood retinol, interferon gamma and zinc in the neonatal life contributed significantly to the development of ESOM. This suggested that development of ESOM might be interplay between quantitative defect in the neonatal immunobiologic factors and the socio-epidemiological variables.

This study established that CSOM was found in a significant proportion (46%) of ASOM. In addition chronicity was greater in MOM than POM, although POM accounted for a higher proportion (55%) while MOM was 45%. Reduced MES levels of IgA, serum levels of IgG and hyporetinolaemia were found to be significant. There appeared to be contributory role of allergy in CSOM and the elevated IgE in the MES suggests a likely local middle ear response. In addition, impaired middle ear immune response was found to be major factor in the nature of secretion. It was high in POM compared to MOM.

6.2. Recommendations

It is recommended that: patients with early suppurative otitis media should have allergy screening and assessment of fetal serum retinol and Zinc at delivery. There is need for further studies on the significance of this difference in relation to the development of acute complications of OM, which have been found to be common in the developing countries. However, assaying for specific immunoglobulin need to be considered in future, as this may further substantiate the result.

Routine retinol and intravenous immunoglobulin supplementation is expected to improve the immunity of these children, thus reduce the likelihood of development of early otitis media among other childhood infections. In addition routine monitoring of the MES immunoglobulin and interferon gamma in children with ASOM may help early detection of those who are at risk of developing CSOM.

The clinical implication of this study is that by assessing the Interferon Gamma level in the middle ear secretion, the doctor can prognosticate the course of the disease and the parents can be better counseled.

CHAPTER 7

7.1. PUBLICATIONS IN SUPPORT OF THESIS (See Appendix 5)

Lasisi O A, Olayemi O and Arinola O G. 2009. Interferon Gamma In Suppurative Otitis Media – Significance in the nature of otorrhoea and outcome of disease. *J Laryngol Otol*. **123**: 1103-1107.

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APPENDIX 1

INSTITUTE FOR ADVANCED MEDICAL RESEARCH AND TRAINING (IAMRAT)

COLLEGE OF MEDICINE, UNIVERSITY OF IBADAN. IBADAN, NIGERIA.

Telefax: 234-2-2412170; 234-2-2410088 / 3594, 3120 Fax: 234-2-2413545

DIRECTOR: *Professor Adeyinka G. Falusi*, B.Sc (Hons), M.Phil., Ph.D.

UI/UCH INSTITUTIONAL REVIEW COMMITTEE

CERTIFICATION LETTER

Principal Investigator: Dr. O.A. Lasisi

IRC Protocol No.:   UI/IRC/02/0009

Protocol Title:

EPIDEMIOLOGY OF CHRONIC SUPPURATIVE OTITIS MEDIA IN NIGERIA.

STATUS : APPROVED

The Joint UI/UCH Institutional Review Committee has received your protocol for a proposed project on "*Epidemiology of Chronic Suppurative Otitis Media in Nigeria.*"

THE RESEARCH PROTOCOL AND CONSENT FORM DESCRIBED ABOVE HAVE BEEN REVIEWED BY THE UI/UCH IRC WITH THE RESULTS AS INDICATED.

27/12/02

Adeyinka G. Falusi   Date
Professor/Chair, UI/UCH IRC
E-mail: uiuchirc@yahoo.com

International Regulations require that any severe drug reaction and unexpected adverse occurrence to subjects during the conduct of this research be reported to the UI/UCH IRC Protocol and Data Management Office promptly. Any changes to this protocol must be submitted for review to the UI/UCH IRC.

Research Units: ■ Medical Genetics ■ Malaria ■ Health & Information Systems ■ Environmental Sciences ■ Epidemiology Research and Service ■ Behavioural & Social Sciences ■ Pharmaceutical Sciences ■ Cancer Research and Services ■ HIV/AIDS ■ Neurosciences ■ Molecular Biology.

APPENDIX 2

Questionnaire On Prenatal Predisposition To Otitis Media (For Pregnant Mothers)

Serial Number_____;

Name_____, Age_____; Sex_____ Gestational

Age _____

Date of delivery_____ Duration of labour_____ Fetal

Weight_____

OM in parents or other children _____

ANTENATAL HISTORY

Antenatal care Y N   Regular visit Y N   Immunization during pregnancy

_____

Fever Y N   Number of episodes of malaria_____ URTI_____

UTI_____   Antenatal hemorrhage_____

Others_____

Hospital admission_____   Drug treatment _____

Fetal cord blood cytokines_____

OM   6 weeks

Ear discharge Y N   Duration of last ear discharge_____

Nature of discharge (mucoid, purulent, serous, watery)

Association of ear discharge with   sorethroat Y N; cough Y N, Recurrence of nasal

catarrh Y N

How many times _____   Duration of last nasal

catarrh_____

EXAMINATION

EAR

Canal – capacious, stenosed, inflamed, discharge, Tympanic membrane - Intact,

retracted, perforated

Middle ear mucosa – inflamed, polypoid, hemorrhagic

Tuning Fork – Rinnes, Weber

NOSE

Allergic hyperpigmentation,   Septum – Midline, deviation, spur,   Mucosal – polyp

THROAT

Diffuse hyperemia,   Tonsils - Inflammed, enlarged, purulent discharge

NEUROLOGIC - Cranial nerves

GENERAL - Allergic skin lesions,

INVESTIGATIONS

Fetal cord Serum: TNF alpha _____   IL – 1 beta _____   IL – 8 ____   Nasal

secretion and Serum Ig E _____

APPENDIX 3

Questionnaire On Immunobiology Of The Ear In Infection And Allergy (Children)

Serial Number_____; Name_____; Age_____; Sex_____

OM

Ear discharge Y N

Tinnitus Y N

Hearing loss Y N

Vertigo/Dizziness Y N

Fullness in the ear Y N

Recurrence of ear discharge Y N

How many times

Duration of last ear discharge

Nature of discharge (mucoid, purulent, serous, watery)

Association of ear discharge with sorethroat Y N; cough Y N

ALLERGY

History of nasal catarrh Y N

Sneezing Y N

Epistaxis Y N

Anosmia Y N

Recurrence of nasal catarrh Y N

How many times

Duration of last nasal catarrh

Any association with a known precipitating factor Y N

known precipitating factors: cockroaches, house dust mites, dogs, mango, grasses and pollens

Any association of nasal and ear symptoms Y N

History of Asthma Y N

Family history of asthma Y N

EXAMINATION

EAR

Canal – capacious, stenosed, inflamed, discharge

Tympanic membrane- Intact, retracted, perforated (size of perforation)

Middle ear mucosa – inflamed, polypoid, hemorrhagic

Tuning Fork – Rinnes, Weber

NOSE

Allergic hyperpigmentation

Septum – Midline, deviation, spur

Mucosal – polyp

THROAT

Diffuse hyperemia

Tonsils - Inflammed, enlarged, purulent discharge

NEUROLOGIC

Balance – Tandem walk, Romberg, Unterberger

Cranial nerves

GENERAL

Allergic skin lesions

INVESTIGATIONS

MEE and Serum:

TNF alpha _____

IL – 1 beta _____

IL – 8 ____

Nasal secretion and Serum Ig E _____

Skin sensitivity test _____

Y - Yes

N – No

APPENDIX 4

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Ag. Director : Prof F. A. A. Adeniyi

UI/UCH INSTITUTIONAL REVIEW COMMITTEE

CERTIFICATION LETTER

Principal Investigator: Dr. O. A. Lasisi

IRC Protocol No: UI/IRC/07/0023

Protocol Title: **IMMUNOBIOLOGY OF THE EAR IN INFECTION AND ALLERGY.**

STATUS: APPROVED

The UI/UCH Institutional Review Committee has reviewed your protocol titled: *Immunobiology of the Ear in Infection and Allergy"*

The study is set out to use the level of the cytokines and quantitative immunoglobulin E to characterize the otitis media (OM) and explore the association with allergy and viral infection in its causation. Findings from the study will assist in understanding the factors involved in development and progression of OM in patients.

THE RESEARCH PROTOCOL DESCRIBED ABOVE HAS BEEN REVIEWED BY THE UI/UCH IRC WITH THE RESULTS AS INDICATED.

O. D. Olaleye
Professor/Chair, UI/UCH IRC
E-mail: uiuchirc@yahoo.com

International Regulations require that any severe drug reactions and unexpected adverse occurrence to subjects during the conduct of this research be reported to the UI/UCH IRC Secretariat promptly. Any changes to this protocol must be submitted for review to the UI/UCH IRC.

Research Units:■Genetics & Bioethics ■Malaria ■Environmental Sciences ■Epidemiology Research & Service ■Behavioural & Social Sciences ■Pharmaceutical Sciences■Cancer Research & Services ■HIV/AIDS .

APPENDIX 5