TO IDENTIFY AND COMPARE MICROBIOLOGICAL PROFILE OF NASOPHARYNX IN CHILDREN WITH FEATURES OF SYMPTOMATIC ADENOID ENLARGEMENT VERSUS ASYMPTOMATIC CHILDREN

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF

THE RULE AND REGULATIONS FOR MS BRANCH – IV

(OTORHINOLARYNGOLOGY) EXAMINATION OF THE TAMIL

NADU DR. MGR MEDICAL UNIVERSITY,

TO BE HELD IN MAY 2022

DEPARTMENT OF OTORHINOLARYNGOLOGY

CHRISTIAN MEDICAL COLLEGE VELLORE

CERTIFICATE

This is to certify that the dissertation entitled "To identify and compare microbiological profile of nasopharynx in children with features of symptomatic adenoid enlargement versus asymptomatic children" is a bonafide original work of Dr. Joyna Singh, submitted in partial fulfillment of the rules and regulations for the MS Branch IV, Otorhinolaryngology examination of The Tamil Nadu Dr. M.G.R Medical University to be held in May 2022.

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DECLARATION

I, Joyna Singh, do hereby declare that the dissertation titled " To identify and compare microbiological profile of nasopharynx in children with features of symptomatic adenoid enlargement versus asymptomatic children" submitted towards partial fulfillment of the requirements of the Tamil Nadu Dr. M.G.R. Medical University for the MS Branch IV, Otorhinolaryngology examination to be conducted in May 2022, is the bonafide work done by me, and due acknowledgements have been made in text to all materials.

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CERTIFICATE – II

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To identify and compare microbiological profile of nasopharynx in children with features of symptomatic adenoid enlargement versus asymptomatic normal children. (Case control study).
Dr. Joyna Singh, PG Registrar ENT 2, Dr Mary John, ENT 2 , Dr Balaji Veeraraghavan, Microbiology, Dr Naveen Kumar, Dr Lydia Jennifer, microbiology, Dr Ajoy Mathew Varghese, ENT 2, Dr. Naina Picardo, ENT 2.

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Dear Dr. Joyna Singh,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Suceena Alexander, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Suceena Alexander
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Dear Dr. Joyna Singh,

The Institutional Review Board (**Blue**, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "To identify and compare microbiological profile of nasopharynx in children with features of symptomatic adenoid enlargement versus asymptomatic normal children. (Case control study)" on December 02nd 2019.

The Committee reviewed the following documents:

1. IRB application format
2. Patient information sheet, Consent and Assent Form (English, Hindi, Tamil, Bengali)
3. Proforma
4. Cvs. Of Drs. Joyna, Ajoy Mathew, Mary John, Naina, Balaji V, Naveen Kumar, Lydiya J.
5. No. of documents 1- 4

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on December 02nd 2019 in the New IRB Room, Christian Medical College, Vellore 632 004.

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We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of Withdrawals for the study entitled: "To identify and compare microbiological profile of nasopharynx in children with features of symptomatic adenoid enlargement versus asymptomatic normal children. (Case control study)" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

The Institutional Ethics Committee expects to be informed about the progress of the project. Any **adverse events** occurring in the course of the project, any **amendments in the protocol and the patient information / informed consent**. On completion of the study you are expected to submit a copy of the **final report**. Respective forms can be downloaded from the following link: http://172.16.11.136/Research/IRB_Polices.html in the CMC Intranet and in the CMC website link address: http://www.cmch-vellore.edu/static/research/Index.html.

Fluid Grant Allocation:

A sum of 1,50,000/- INR (Rupees One Lakh Fifty Thousand Only) will be granted for 2 years.

Yours sincerely,

Dr. Suceena Alexander
Secretary (Ethics Committee)
Institutional Review Board

Dr. Suceena Alexander, MD.,DM.,FASN.
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IRB Min. No. 12412 [OBSERVE] dated 02.12.2019

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I want to offer this endeavour to our God Almighty for the wisdom He bestowed upon me, the strength, peace of mind and good health in order to finish this research. I would like to express my gratitude towards my family for the support and encouragement which helped me in completion of this thesis.

CONTENTS

ABSTRACT

To study and compare the microbiological profile of nasopharynx in children with symptomatic adenoid enlargement and asymptomatic normal children

BACKGROUND

Adenoids, an aggregate of lymphoid tissue situated in the nasopharynx is commonly enlarged in children between the age of two to eight years due to the normal physiological growth pattern. Hypertrophied adenoid can also act as a focus of infection with can lead to ear, sinonasal and repeated respiratory infections. In some children enlargement of adenoids can cause obstructive symptoms including sleep disturbances. The nasopharyngeal microflora consists of pathogenic and commensal bacteria which undergo constant interaction and along with other factors results in destruction of pathogenic bacteria or disease production. Therefore knowledge of the microbiological profile of adenoids in symptomatic and asymptomatic children is relevant. A better understanding of the pathogenic and commensal bacteria and biofilm forming capacity of pathogenic bacteria is the broad theme of this study.

METHODOLOGY

The study was a prospective comparative study where 90 children were recruited, 60 in study arm and 30 in control arm . The study group consisted of children with symptoms of adenoid enlargement and control group had asymptomatic children. The history and

clinical presentation of each child was documented following which adenoid size was assessed by Rigid nasal endoscopy and or X ray lateral view of nasopharynx. Nasopharyngeal swab was taken after that and porcessed in Department of Microbiology for evaluating nasopharyngeal microflora.

Microbiological assessment consisted of detecting the growth of pathogenic or commensal bacteria from the nasopharyngeal swab and semi quantifying it. This was followed by the study to detect the ability of pathogenic bacteria to form biofilm. The strength of biofilm formation and the growth of each pathogenic bacteria on the biofilm was also studied.

RESULT

In both study and control group, the predominant microflora was commensal and was present in 70% and 66% of individuals respectively. The most common pathogens in both the group were Staphylococcus aureus and Streptococcus pneumonia. As age progressed, a decreasing trend in the number of commensals and replacement by pathogenic bacteria was observed. Pathogenic bacteria in study and control group had 68% and 55% capacity to form biofilm respectively. Staphylococcus aureus was associated with strong biofilm forming capacity, followed by Streptococcus pneumonia.

CONCLUSION

This study compared the microflora of nasopharynx in asymptomatic children and children with adenoid hypertrophy. It helped to gain a better understanding of the changes of microflora in the nasopharynx of a child with advancing age. Early childhood is associated with more commensals in the nasopharynx even in children with adenoid hypertrophy. Biofilm forming capacity of the pathogenic bacteria were also studied.

INTRODUCTION

The adenoids are a part of the Waldeyer's ring and play an important role in the development of the immune system and in the defense against infection. Adenoid enlargement is commonly seen in children between the age of two to eight years particularly due to the normal physiological growth pattern and also due to associated factors like allergy.

Children presenting with complaints like nasal obstruction, mouth breathing, snoring and ear ache arises the suspicion of possible adenoid enlargement. Though adenoid enlargement is physiologically normal in this age group, the resulting airway obstruction can cause distressing symptoms in the child. Adenoid hypertrophy can also cause eustachian tube dysfunction leading to otitis media with effusion which if not treated adequately may result into a chronic discharging ear. Chronic adenoiditis can act as a focus of infection with repeated respiratory infections. This further leads to poor appetite in the child resulting in poor nutritional status. Lack of good sleep due to obstructive symptoms leads to poor concentration and poor school performance.Previous studies have shown that adenoid hypertrophy causing chronic airway obstruction can lead to obstructive sleep disorders.

Many studies have reported that in children having adenoid hypertrophy and chronic adenotonsillitis, both harbour aerobes and anaerobes. The aerobic organisms most frequently isolated in both groups of children were *alpha- and beta-hemolytic Streptococci, Staphylococcus aureus, beta hemolytic Streptococci, Hemophillus* species. The predominant anaerobic organisms in both groups were *Bacteroides* species, *Fusobacterium* species,anaerobic gram positive cocci.

"Biofilm can be defined as a sessile community consisting of cells that are attached to an interface or to each other, embedded in an extracellular polymeric matrix that is produced by them and demonstrate an altered phenotype associated with a differential gene expression rate" . An organized biofilm includes adherence of the microorganisms either to a surface or to each other by quorum sensing, a change in gene expression resulting in a different phenotype from the planktonic state and an extracellular matrix that is composed of host components and secreted by the bacterial products.

H. influenzae, *S. pneumoniae*, *M. catarrhalis*, *S. aureus* pathogenic *streptococci*, *Pseudomonas aeruginosa*, *Neisseria meningitidis*, *Fusobacterium nucleatum* are some of the commonly isolated organisms from the biofilm over the surface of adenoids. Biofilms consisting of unidentified bacteria have also been found on the surfaces and crypts of adenoids removed from children being treated for COM, CRS,chronic adenotonsillitis. Biofilm infections are clinically significant because these three-dimensional (3D), adherent, organized communities of bacteria are far more recalcitrant to antibiotic therapy and killing by host phagocytic cells.

Knowledge of the microbiological profile of adenoids in symptomatic and asymptomatic children is relevant. Hence a better Understanding of the commensal bacteria and biofilm forming pathogenic bacteria and is the broad theme of this study.

AIM AND OBJECTIVES

Aim

The aim is to study the microbiological profile of nasopharynx in children with symptomatic adenoid enlargement and asymptomatic normal children

Objectives of the study

- To compare the microbiological profile of nasopharynx in children below 16 years with symptomatic adenoid enlargement and asymptomatic normal children
- To determine the biofilm forming capacity of the organisms in the symptomatic group.

REVIEW OF LITERATURE

Introduction and epidemiology

Adenoid is a collection of lymphoid tissue present in the nasopharynx which forms a part of Waldeyer's ring. Adenoid hypertrophy is defined as an enlargement of adenoids, which may be simple or inflammatory (1). Adenoid enlargement is considered as one of the most common cause of nasal obstruction (2–5).

Adenoid has its growth spurt between the ages of three to ten years and starts to decrease by the age of 15. Also, children who presented to ENT clinic with features of sleep apnea, the prevalence of adenoid enlargement in such children ranged from 42-70% (3). Another study done by Isaac et.al amongst the children belonging to age group of 7-14, the prevalence of adenoid hypertrophy was found to be 41% (6).

Development of adenoid

During embryological development, lymphoid tissue can be seen at 4-6 weeks of gestation, present within the mucous membrane of the roof and posterior wall of nasopharynx (7). The adenoid is a lymphoid organ that essentially develops from three sources: First, an epithelial component arising from the lining of the primitive oronasal cavity. Second, this epithelium grows into and is enveloped by a connective tissue or mesenchymal stroma. Third, the region is infiltrated by lymphoidal cells. The resultant organ is composed of resident population of lymphoid cells in association with a more or less elaborated epithelial framework (the crypts) that has grown into, and been enveloped by, mesenchymal tissue. The adenoid develops in close association with mucous glands. As early as the third month of development, glandular primordia are visible as solid buds or cords of cells surrounded by blood vessels and an increasing number of discrete lymphoid cells in a loose mesenchyme. During the fourth month, the lymphatic vessels appear and glandular primordia increase in number and complexity as they branch and acquire lumen and show evidence of secretary activity. Infiltration by lymphoid cells is intense. In the fifth month, pharyngeal crypts appear as 12 shallow sagittal folds or plicae . These folds are covered with pseudostratified ciliated epithelium with goblet cells. Discrete lymph follicles organize around the glandular ducts, and lymphocytes penetrate

the epithelium and into the adenoid crypts and lumen of the nasopharynx. The adenoid folds deepen during the sixth month and form fully developed tonsil during the seventh

month. The alveoli of the glands come to lie deep to the lymph follicles. The peculiarly dilated glandular ducts pass through, or next to, the lymphoid follicles. Further evidence of function of these tissues was reported in a study which, demonstrated that IgA, IgG and IgM were all present in epiphyaryngeal tissues taken from 5 to 16 week old human embryos

Anatomy of Nasopharynx

Pharynx is a conical neuromuscular tube extending from base of skull to inferior border of cricoid cartilage anteriorly and inferior border of C6 vertebra posteriorly. Nasopharynx forms the upper part of pharynx that lies behind the nasal cavity extending from base of skull to nasopharyngeal isthmus (8) and is lined by respiratory epithelium (9)

The nasopharynx communicates with the nasal cavity anteriorly through the posterior nasal aperture or choana. Posteriorly it is bounded by the prevertebral fascia and the roof is formed by body of sphenoid and basiocciput. Inferiorly its bounded by soft palate anteriorly and is deficient posteriorly to be connected to the oropharynx.

The lateral wall of nasopharynx receives opening of the Eustachian tube and forms a communication with the middle ear. The mucosal elevation above and behind the tubal opening is called torus tubarus. Posterior to this lies Fossa of Rosenmuller.

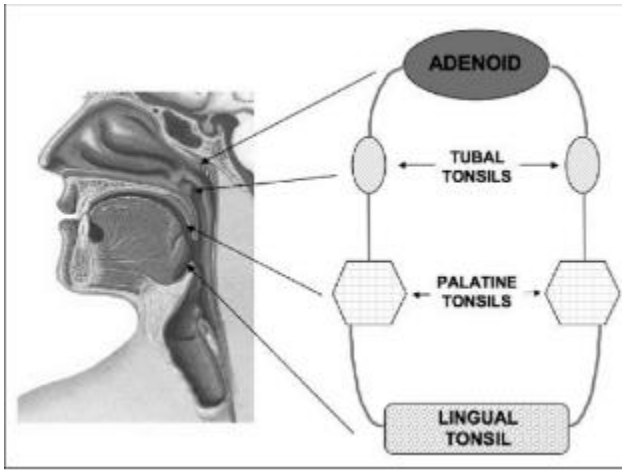

Figure 1: Waldeyer's Ring

The pharyngeal, palatine and lingual tonsils together form the so called Waldeyer's ring .The ring is a group of sub epithelial lymphoid aggregates at opening of oropharynx and nasopharynx to external environment. The adenoid is composed of mucosa associated lymphoid tissue (MALT) located at the junction of the roof and posterior wall of the nasopharynx. During the early years of life, it is truncated pyramid in shape, often with a vertically oriented median cleft, thus its apex points towards the nasal septum and its base at the junction of the roof and posterior wall of the nasopharynx. The free surface of the

nasopharyngeal tonsil is marked by folds that radiate forwards and laterally from a median blind recess, the pharyngeal bursa (bursa of Luschka), which extends backwards and up.

Blood supply

Nasopharynx receive its Blood supply from ascending pharyngeal artery, ascending palatine artery, Pharyngeal branch of internal maxillary artery, artery of pterygoid canal and contributions from tonsillar branch of facial artery

Venous drainage from the adenoid is through the pharyngeal plexus which in turn drain into the internal jugular vein (8)

Nerve supply

Adenoid is supplied by sensory branches of the glossopharyngeal and vagus nerve (10)

Lymphatic drainage

Lymphatic drainage is to the retropharyngeal lymph nodes, upper deep cervical nodes particularly in the posterior triangle of the neck

Physiology of nasopharynx

Nasopharynx serves as a pathway for air passage. It is separated completely from oropharynx by Passavant's ridge,which is a prominence by contraction of superior constrictor's upper fibres, against soft palate. The mucous secretion comes from the nose through the nasopharynx into oropharynx. . Enlargement of adenoid tissue further limits the nasopharyngeal airway and may result in obstruction

Immunology of adenoid

Adenoid is a part of Waldeyer's ring. Waldeyer's ring is a ring of lymphoid tissue consisting of adenoid, palatine tonsils, lingual tonsil, scattered lymphoid follicles, lateral pharyngeal bands, and tubal tonsils near the Eustachian tube (11).The lymphoid tissue has only afferent lymphatic channels (12)

The primary function of these tissue is to form antibodies (13). Exposure to antigens during early childhood leads to formation of these antibodies as part of natural immunity. They form B cell , immunoglobulin and T lymphocytes which is involved in cell mediated or delayed immunity.

The adenoids and tonsils are B cell organs and accounts for around 50-65% of all the lymphocytes (14).The lymphoid cells that takes part in immunological reaction are found

in four areas : mantle zone of lymphoid follicle, the reticular cell epithelium, the germinal centre of the lymphoid follicle and extra follicular area (15)**.**

Even though there are six different groups of lymphoid tissues, the major immunogical reaction takes place in the tonsil and adenoid. Intratonsillar defense mechanism removes weak pathogenic signals, and only higher concentration of antigen leads to development of antigen sensitive B cells in the germinal layers (16) . This step of generation of B cells in germinal layers is considered the most essential tonsillar function (17). In case of repetitive exposure, the inflammation of epithelium leads to shedding of immunologically active cells and decreased antigenic function and subsequent replacement of germinal centers by stratified squamous epithelium(15) (18).

Adenoids and tonsils are favourably located to mediate immunologic protection of the upper aero digestive tract as they are exposed to airborne antigens. The generation of B-

cells in the germinal centers of the tonsils and adenoids is considered to be one of the most essential functions. Immunoglobulins (Igs) produced by the adenoid include IgG, IgA, IgM and IgD. IgG appears to pass intT-cell functions such as interferon production and presumably production of other important lymphokines have been shown to be present in tonsils and adenoids.

The role played by tonsillar and adenoid T cells in tumour response is still unknown. The human tonsils and adenoid are immunologically active between the ages of 4 and 10 years. Involution of tonsils begins after puberty, but involution of adenoids starts by 8 years of age. This involution results in a decrease of B-cell population and a relative increase in the ratio of T to B cells. Although the overall Immunoglobulin producing function is affected, considerable B-cell activity is still seen in clinically healthy tonsils even at 80 years of age. The situation is different in disease associated changes, such as when recurrent tonsillitis and adenoid hyperplasia are observed. Inflammation of the reticular crypt epithelium results in shedding of immunologically active cells and decreasing antigen transport function with subsequent replacement by stratified squamous epithelium. These changes lead to reduced activation of the local B-cell system, decreased antibody production, and an overall reduction in density of the Bcell and germinal centers in extrafolliuclar areas of the nasopharyngeal lumen by passive diffusion.

Histology

The surface of adenoid is covered by pseudostratified respiratory epithelium. The surface epithelium forms crypts which increases the surface area and enhances antigen

interaction. The lymphoid nodules underlying the epithelium has germinal centers and plenty of B and T cells and macrophages (12)

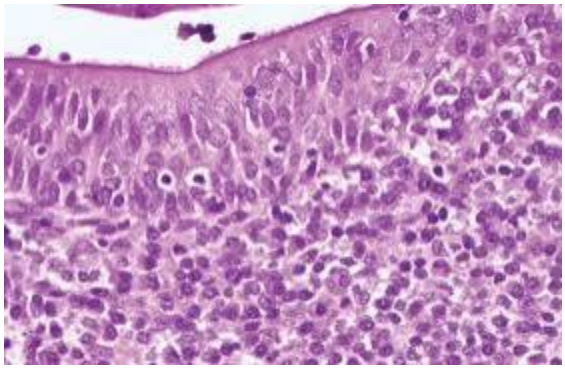

Figure 2: Histology of Adenoid

Diseases of adenoid

Adenoid enlargement can be associated with various upper respiratory tract diseases due to the obstruction of the nasal choana and also as result of infections.

Otitis media with effusion

This condition is also known as glue ear and is characterized by accumulation of fluid behind intact drum. It is commonly seen in children in the first decade of life. Spontaneous resolution occurs in around 25-30 % of individuals (14) with a recurrence rate of around 50% within 24 months. Otitis media with effusion (OME) causes conductive hearing loss which is temporary in the beginning, but recurrent episodes can lead to significant hearing loss. This in turn can have an adverse effect on child's speech development and social behavior (19–22).

Treatment options available can be surgical and non surgical. Since adenoid enlargement has a role to play in the pathogenesis of OME, adenoidectomy is considered along with grommet insertion (23,24).

Recurrent otitis media

Dysfunction of Eustachian tube plays an important role in the development of recurrent otitis media. The dysfunction in turn is caused by mechanical obstruction as a result of enlarged adenoids. Due to the presence of adenoids, the nasopharyngeal opening of Eustachian tube is blocked hence leading to a development of negative pressure in the middle ear cavity which in turn causes mucosal transudation (25). In children with otitis media, adenoid enlargement is frequently observed (26–28).

Upper airway obstruction and sleep breathing disorder

Sleep disordered breathing refers to disorders that occur or are exacerbated during sleep. The cause can be either central, apnea of prematurity or obstructive disorders. Children with obstructive causes presents with symptoms such as snoring, sleep fragmentation and excessive daytime sleepiness (4,29) . Those who have mild form of obstructive sleep apnea (OSA) present with behavioral disturbances such as emotional irritability, aggression ,hyperactivity (30) and cognitive impairment. More severe forms of OSA are at risk for failure to thrive, hypertension and cardiac failure. The incidence of OSAS is estimated to be 2% where as snoring is estimated to occur in 6-9% in children (31,32) . The age at which maximum symptoms are seen, correlated well with the time when growth of lymphoid tissue occured. Hypertrophy of adenoid and tonsils are one of the most common causes of SDB in children (29,33). It causes narrowing of airway leading to partial or complete obstruction, mainly during sleeping when the pharyngeal muscle relaxes. The treatment for such cases includes adenoidectomy (33,34). Post adenoidectomy, studies have shown significant improvement in quality of life, polysomnographic findings and behavioral pattern as compared to the ones who did not undergo surgery(33) .

Rhinosinusitis

Children with allergic rhinitis have a greater susceptibility to adenoid hypertrophy than non allergic children, the possible reason being IgE mediated inflammation of nasal mucosa and adenoids (35).

Allergic Rhinintis

Allergic rhinitis is a common disorder which is seen commonly in children and adolescents. This could be associated with a spectrum of other allergic diseases like asthma, eczema. It is characterized by classical symptoms of sneezing, nasal itching, nasal obstruction and rhinorrhea.

Allergic rhinitis and adenoid hypertrophy are quiet often associated with each other. There have been studies concluding that there were improvement in respiratory symptoms and reduction in adenoid size after anti allergic medical therapy. However there was not much improvement seen adenoidectomy with regards to allergic symptoms

Evaluation of Adenoid hypertrophy

Symptoms

1) Nasal obstruction: This is the most frequently reported symptom. Nasal obstruction may be associated with symptoms like mouth breathing, snoring, nasal discharge, apneic episodes while sleeping, hyponasal voice, day time somnolence and enuresis.

2) Throat pain / dysphagia : In most of the cases adenoiditis is usually associated with tonsillitis. The patient may complain of throat pain and dysphagia. Pain may radiate to the ears or may occur in the neck due to enlargement of the jugulo digastrics lymphnodes. Swallowing is acutely sore and solid food is refused at the height of the inflammation although fluids are usually accepted.

3) Otalgia/Otorrhoea : Very young children will not complain of pain but will be irritable .Conversely some children may become very quiet and refuse food, not sleep well or be inconsolable. If the ears discharge ,it is usually mucopurulent.

Signs

There is a characteristic facial appearance in children with adenoid hypertrophy .The so-called "adenoid facies" has classical appearance that include :

a) Open mouth

b) Crowding of upper teeth

c) Short upper lip

d) High arched palate

e) Pinched nose

f) Hypoplastic maxilla

g) Narrow alveolus

Figure 3: Adenoid facies

Adenoid enlargement can be assessed using X ray lateral view of nasopharynx, acoustic rhinometry and rhinomanometry , posterior rhinoscopy , video fluoroscopy ,or endoscopic evaluation (36).

In X ray nasopharynx , the size of the adenoids can be graded based on Cohen and konak's grading system (37)or Fujiokas method which takes into account the adenoid/nasopharyngeal ratio (38).

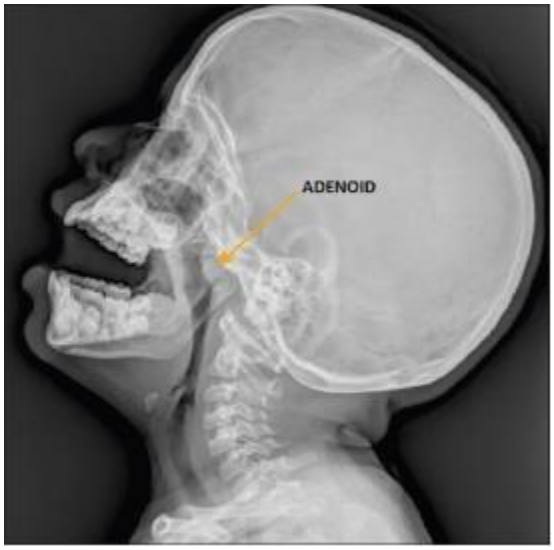

Figure 4: X Ray lateral view of Nasopharynx

The advantage of using X ray is that it is economical, easily available, non invasive and comfortable for the child (39). However the main disadvantage being exposure to radiation (40,41).

Similary in endoscopic evaluation Clemens classification can be used to grade the adenoid enlargement (42). Various studies done in the past comparing the X ray and Endoscopy found that Flexible endoscopic evaluation was more sensitive in assessing the size of the adenoids. Kindermann et al study found the sensitivity and specificity of flexible endoscopy to be 925 and 71 % respectively (43). Similar studies done by Ehab Yaseen and Yogita Dixit concluded that Flexible endoscopy is better is assessing the size of adenoid enlargement when compared to X ray.

A.C.E. grading system

This grading system is termed after the variables it measures.

A - Adenoid size	C - Choanal obstruction	E - Eustachian tube
0 - No adenoids	0 - No obstruction	0 - Not abutting Eustachian tube
1 - 1% to 25%	1 - <50% obstruction	1 - Abutting Eustachian tube
2 - 26% to 50%	2 - >50% obstruction	
3 - 51% to 75%		
4 - 76% to 100%		

Figure 5: A.C.E Endoscopic grading of adenoid enlargement

It is a safe method which provides a three dimensional view of the nasopharynx and is more accurate in measuring the size of adenoids and degree of choanal obstruction (40).

However one major disadvantage is that it is invasive and is not well tolerated by younger age group.

Management

Treatment of symptomatic adenoid enlargement consist of medical therapy and definitive surgical therapy

Medical

Medical management consist of use of intranasal corticosteroids in children with symptoms suggestive of airway obstruction due to adenoid hypertrophy (26,44–47) . Amongst the intranasal steroids mometasone fuorate is the most commonly used in pediatric population above 2 years of age due to its safety and benefit . It is beneficial in approximately 50-70% of individuals and helps in significant reduction in size of the adenoids thereby providing symptomatic relief (44,47,48).

The use of steroid have also shown improvement in symptoms of allergic rhinitis.

Surgical

It is the definitive treatment for adenoid hypertrophy, however it can have a negative influence on immunological system (49,50)

Adenoidectomy

Indication (51–53)

Airway obstruction

Obstructive sleep apnea syndrome

Speech impairment

Eating and swallowing disorders

Abnormal dentofacial growth

Halitosis

Recurrent otitis media

Recurrent or chronic rhino sinusitis or adenoiditis

Contraindications for adenoidectomy

Overt cleft palate §

Submucous cleft palate §

Haemorrhagic diathesis §

Acute infection of upper respiratory tract

Procedure

Procedure is done under general anesthesia

Position: Rose's position where the patient lies supine with a pillow under the shoulder for head extension and a head ring to stabilize the head.

Steps of operation:

Boyel Davis mouth gag is inserted to visualize the oropharynx.

Examine the nasopharynx by gently retracting the uvula and soft palate and by digital palpation. The size of the adenoid can therefore the assessed at this point. Adenoid curette with guard is introduced into the nasopharynx till its free edge touches the posterior border of nasal septum and then is pressed backwards to engage adenoids. Head is flexed gently to avoid injury. With gently sweeping movement, adenoid is shaved off. This step is repeated to clear off any adenoid tissue laterally. Homeostasis is achieved by packing the nasopharynx for some time.

Complication

Significant complications post procedure is uncommon in children

1) Hemorrhage: The bleeding can happen during the procedure or post procedure from the nose which mostly happens within 24 hours. This is called primary or early hemorrhage. Late or secondary hemorrhage is rare (54–56).
2) Injury to Eustachian tube opening
3) Injury to pharyngeal musculature and vertebrae

4) Griesel syndrome : Atlantoaxial subluxation or griesel syndrome is a non traumatic subluxation of the atlantoaxial joint. Main symptoms include severe neck pain followed by torticollis and pain on neck movements. The use of monopolar cautrey is a risk factor.

Children with Down syndrome are at higher risk of this complication as there is increased laxity of their cervical ligaments (57). Early identification to prevent progressive neurological squeal from spinal cord injury if important .

5) Nasopharyngeal scarring: Nasopharyngeal stenosis following scarring is a rare complication following adenoidectomy. It is characterized by significant narrowing or obliteration of the passage between nasopharynx and oropharynx. Children presents with breathing difficulty, hyponasal speech and dysphagia. In severe case of stenosis obstructive sleep apnea, chronic rhinorrhea, and anosmia can be seen. Treatment mainly involves surgical correction.
6) Recurrence: Regrowth of adenoids and recurrence of symptoms due to incomplete removal of adenoid , especially when older technique of adenoidectomy like curettage without visualization is performed. The incidence of repeat surgery due to recurrent symptoms is estimated to be 0.55-3%.

Microbial flora of nasopharynx

Upper respiratory tract acquire complex microbial community with both commensals and pathogens as a part of microbial flora. Adenoids thus being a part of upper respiratory tract (URT) has a diverse microbial community (58).

A study carried out by Brook and Bethesda to study the microbiological profile of adenoid included 18 children with adenotonsillitis (group A) and 12 children with adenoid hypertrophy(group B) in the age group from 20 months to 15 years. In the 30 sample that was studied 138 aerobes and 97 anaerobes were isolated.

Common aerobes in both the group included *alpha* and *gamma haemolytic streptococci, beta haemolytic streptococci* group A,B,C,F, S.*aureus, Haemophilus* sp, *S. pneumonia*(59)

Common anaerobes cultured in both groups included *Bacteroid sp, Fusobacterium Sp,* Anaerobic gram positive cocci . Group A in addition also had *B. fragilis* and Veillonella *parvula*(59)

In another study done by Rajeshwari et at, 100 children aged between 3-2years were included in the study based on clinical features , examination findings and X ray nasopharynx soft tissue neck to determine the bacteriology of symptomatic adenoid. Adenoid core tissue and surface swab were taken for microbiological analysis post adenoidectomy. They found that 97% specimen had aerobic growth and 63% showed anaerobic growth , whereas 7 % specimen had no growth.

Common gram positive aerobic pathogenic species found were *S. aureus, Streptoccus pneumonia, group A beta haemolytic streptococci and enterococcus*. Commensals isolated were *S.pyogenes, S.epidermidis, S.viridans*. Gram negative aerobic species were *Pseudomonas, Klebsiella, E.coli, H. influenza* and *Moraxella* being the commensal.

Anaerobic organisms included *Peptostreptococcus, Prevotella, Fusobacterium and Bacteroids*(60)

Brook and Shah conducted a study which looked at the bacteriology of adenoid and tonsil in recurrent adenotonsillitis in children aged between 4-15 years who underwent adenotonsillectomy. Core tissue was sent for microbiological analysis in which 111 aerobes and 118 anaerobes were isolated. Aerobic organism frequently isolated were *Alpha Haemolytic streptococci, S. aureus, Beta hemolytic streptococci* group A,C,E, *Gamma hemolytic strep, Haemophilus sp and moraxella catarrhis.*

Anaerobes were *Prevotella sp, Bacteroid fragillis, Fusobacterium sp, gram positive cocci and veillonellaparvula* (61)

The result of above mentioned studies were similar to the outcome of study done by Khalid et.al where the common organisms colonising the adenoids were found to be the same (62).

Brook in 2016 studied the effects of antimicrobial therapy on microbial flora of adenoids stated that the core normal adenoid harbours aerobic, anaerobic and potential respiratory pathogens(59,63). Number of bacteria in the tissue was between 10^3-10^6/gram. These aerobic and anaerobic bacteria are capable of interfering with the growth of pathogenic bacteria(64,65).Inflamed or hypertrophied adenoids had similar bacteria but in higher number and frequency(61) and the commonest bacteria found were *S.pyogenes, S.aureus, H.influenza, S.pneumoniae* (1,66). He studied bacterial profile of 60 children who underwent adenoidectomy and found the commonest aerobes were *alpha and gamma haemolytic streptococci, H.influenza, S.aureus, S.pyogenes, M.catarrhalis* and anaerobes were *Peptostreptococci, Prevotella, Fusobacterium*(1)

Brodsky et al studied quantitative and qualitative aerobic flora in 69 children undergoing adenoidectomy for OHA with 16 controls and found that only 4 controls grew pathogenic bacteria, compared to half of other adenoids. *H.Influenza* was found in 50 % of diseased adenoid as compared to 19% of controls (67).

All the above mentioned studies mostly compared the bacterial profile of hypertrophied adenoid and compared it with samples obtained from children who had adenoids or adenotonsillitis.

Pathogenic bacteria

Pathogenic bacteria can be classified as aerobic and anaerobic.The commonly encountered organisms in the nasopharynx are described below**.**

Staphylococcus aureus are gram positive cocci which are non capsulated, non motile and non sporing. It is coagulase and catalase positive. Various skin infections like carbuncle, furuncle, wound infection and systemic infections such as pneumonia, arthritis, sepsis etc is caused by staphylococcus. This organism can be cultured in aerobic environment using nutrient blood or Macconkey agar. It is known to release toxins like toxic shock syndrome toxin, exfoliative toxin, hemolysin and leukocidins.

Streptococcus pneumonia are gram positive cocci arranged in pairs, are capsulated, non motile and non sporing. It ferments sugar producing acid and is catalase and oxidase negative. It is responsible for clinical diseases like pneumonia, meningitis, sinusitis and otitis media. Bile solubility test, optochin sensitivity test are few of the tests to identify the organism

Group A beta haemolytic streptococci are gram positive cocci seen in short chains and produces a clear zone of hemolysis on blood agar. They are responsible for infections like bacterial pharyngitis, cellulitis, scarlet fever, glomerulonephritis and rheumatic heart disease. Biochemical reactions to identify the organism are- it is catalase negative, ferment sugars and is not soluble in bile.

Enterococcus are gram positive oval or spherical coccobacillary which are seen in pairs or short chains. They are non mobile and non capsulated. They are usually non pathogenic but are recently emerging as agents of nosocomial infections.

Pseudomonas are straight or slightly curved gram negative bacilli which are arranged singly, in pairs or in short chains. It possess a flagellum and are therefore mobile. It shows growth in nutrient Macconkey agar and nutrient broth. It is oxidase and catalase positive. They are found to be causative factors in respiratory tract infections, skin and urinary tract infections.

E. coli- *E.coli* is both a commensal and pathogen. It is a gram negative bacillus, arranged singly or in pairs. Mobile and capsulated . It can be cultured on medium like nutrient agar and Macconkey agar. It releases shiga toxin, heat stable toxin and exotoxins responsible for a spectrum of diseases such as urinary tract infection ,gastroenteritis, HUS, neonatal sepsis.

Klebsiella are gram negative rod shaped, non motile bacteria with a prominent polysaccharide capsule. They are lactose fermenting, indole negative organisms that produce red colonies on macconkey agar. They possess many virulence factors like capsule, multiple adhesions which plays a role in development of infectious diseases like community acquired pneumonia, nosocomial infections, bacteremia and sepsis.

Haemophilus influenza is a small, pleomorphic, gram negative bacillus which is non motile, non sporing and non acid fast. It grows well on chocolate agar and exhibits satellitism on blood agar. It is catalase and oxidase positive. Main virulence factors are capsular polysaccharide and IgA1 protease. Infections associated are meningitis, epiglottitis, cellulitis, pneumonia, otitis media and bronchitis.

Anaerobic bacteria commonly seen in the nasopharynx are :

Fusobacterium- Gram negative bacilli surrounded by a polysaccharide capsule. They are fastidious and grows slowly on culture media.

***Bacteroid*-** Gram negative bacilli surrounded by polysaccharide capsule which is antiphagocytic in nature hence allowing bacteria to adhere to tissue without getting destructed. They mainly cause head and neck soft tissue infections, intra abdominal and bacteremia.

***Peptostreptococci*-** Anaerobic gram positive cocci which are seen as normal inhabitants of oral cavity and gastrointestinal tract. They can be cultured on blood rich agar.

Prevotella - gram negative bacilli causing head and neck, gynecological infections.

Commensal

Commensalism is a type of biological interaction in which member of one species gain benefits while those of other species are neither harmed nor benefitted.

Commonly found commensals in nasopharynx of humans are described below.

***Moraxella*-** They are gram negative cocci arranged in pairs, non motile, non capsulated and nonflagellated bacteria. Oxidase and catalase positive. They are obligate aerobe and produce small colonies on blood agar. M.catarrhalis is found as a normal commensal in human respiratory tract. But can cause bronchopneumonia and bronchitis in elderly.

Streptococci viridians- They are a heterogenous group of alpha haemolytic and non haemolytic organisms. They are found as commensal flora in oropharynx, oral cavity, gastrointestinal tract and urinary tract. Fastidious organisms which grows well on blood agar facilitated by presence of carbon dioxide .

Staphylococcus epidermidis – They are coagulase negative staphylococcus which are normal flora of the skin and can be found in nasopharynx as a commensal. They cause infection in debilitated or immunocompromised patients and in patients with cardiac valve, catheters or pacemakers. Carriage rate is as high as 100% and is transmitted by self inoculation or contact with infected person.

Streptococcus pyogenes- It is classifies as group A streptococci, gram positive cocci arranged in chains , non motile, non sporing. It ferment sugars and are catalase negative. Growth is seen on blood agar. It can cause infections like pharyngitis, pyoderma, scarlet fever, toxic shock syndrome, rheumatic fever and acute glomerulonephritis.

Effect of antibiotics on nasopharyngeal flora

The nasopharynx of normal children is colonized by various aerobic and anaerobic organisms. Administration of antibiotics can affect the composition of nasopharyngeal bacterial flora (1).

A study done by comparing the effect of administration of amoxicillin and augmentin on nasopharyngeal flora pointed out that there was a significant reduction in the number of isolated recovered post antimicrobial therapy

The potential pathogenic organisms like *S.pneumonia, S.aureus, B-haemolytic streptococci, Haemophilus, M.catarrhalis* after therapy in augmentin group was lower than the ones treated with amoxicillin alone(68,69).

Another study conducted to compare the effect of amoxicillin, clindamycin on nasopharyngeal flora revealed that polymicrobial aerobic-anaerobic flora were present in all the cases pre therapy. . The predominant aerobes in all groups *were alpha hemolytic* and *gamma-hemolytic streptococci, Haemophilus influenzae, Staphylococcus aureus, group A beta-hemolytic streptococci, and Moraxella catarrhalis*. The prominent anaerobes were *Peptostreptococcus, Prevotella, and Fusobacterium spp*.

The number of isolates was significantly reduced in those treated with Amoxicillin (70).

Nasopharyngeal swab

For microbiological analysis of organisms in the nasopharynx, a swab is taken from the nasopharynx. Flocked nylon swabs have protruding nylon fibers that improve the recovery of target organisms from the sampled surface, and allow for the rapid transfer of collected material into the transport medium.

The process of sample collection is described below:

Hold the infant or young child's head securely. Tip their head backwards slightly and pass the swab directly backwards, parallel to the base of the NP passage. The swab should move without resistance until reaching the nasopharynx, located about one-half to two-thirds the distance from the nostril to ear lobe(71) . The sensitivity of a single swab was 95% (1).There was no evidence of advantage to swabbing either the right or left nostril .

Figure 6 : Nasopharyngeal Swabbing

Biofilm

Biofilms are multidimensional communities in which resident bacteria coexist within the self-derived extracellular matrix (ECM) (72,73). Although the initial developmental stages which leads to biofilm formation are conserved, every species forms a unique multicellular community (74,75). This formation possesses the ability to withstand environmental threats such as antimicrobials and host defense mechanism (76).

Biofilms have been found to be ubiquitous . Biofilms are being produced in the harshest environments like in hot springs and deep-sea vents, on rocks and soil, the roots and stems of plants, on chitinous surfaces of aquatic animals, many manmade objects as well.

Biofilms account for 80% of chronic microbial human infections, leading to increased rates of hospitalization, elevated health care costs, and increased mortality and morbidity rates (77). Upper and lower respiratory tract diseases, native valve endocarditis, chronic otitis media, eye infections, chronic wounds, diabetic foot ulcers, urinary tract infections (UTIs), and periodontitis are all biofilm-associated diseases (78,79). Biofilms can also develop on abiotic surfaces, including medical devices such as orthopedic prostheses, artificial cardiac valves, coronary stents, intravascular and urinary catheters, neurosurgical, cochlear, and breast implants, dentures, and ventricular-assist and ocular devices(80)

The ECM typically includes polysaccharides, proteins, and/or DNA (81). However, the ECM structure can be differentiated based on the following factors :-

1) Species or strains comprising the biofilm (82,83);

2) Conditions during development, and in turn the expression of bacterial factors (84)

3) Spatial location sampled within any given biofilm (85).

The ECM components affect structure, physiology, interactions with the surrounding environment, resistance toward antibiotics, and host defense mechanisms (86). Bacteria can build exposed or submerged biofilms on either biotic or abiotic surfaces. This happens under static or shear-flow conditions or, alternatively, coalesce directly in the host, as seen in intracellular bacterial communities (IBCs) involving uropathogenic *Escherichia coli* (UPEC) and *Klebsiella pneumonia* (87–90).

Surface-Associated Biofilms

Surface-attached biofilms forming colonies are the ones that are formed over a solid surface. Different gene expression patterns can be seen by comparing biofilms attached to solid surfaces and planktonic bacteria grown in liquid cultures (91–96). Surface-associated biofilms are highly dependent on the substratum material and may or may not

be exposed to air. Among the most common materials that promote biofilm formation on abiotic substrata are polyvinyl chloride (PVC), silicone, polystyrene, and metal (97,98).

Biofilm and Phenotypic Resistance

The biofilm life cycle ends with the dispersal of bacterial cells stemming from the biomass after maturation (99). The dissemination and colonization of new site leads to chronic infection . Biofilm-originating cells form bacterial niches with resistance phenotypes at the newly colonized sites. The biofilm dispersal process is controlled by environmental signals (oxygen, nutrients, temperature, and signaling molecules), intracellular reduction of the concentration of c-di-GMP, and upregulation of motility or quorum sensing (QS) genes, though many bacterial dispersal signals remain cryptic (100,101).

Since the environmental gradients surrounding the microbial community influence biofilm composition, phenotypically distinct subpopulations arise, including extracellular matrix producers, adhesive fiber producers, motile bacteria, and metabolically quiescent and/or antibiotic-tolerant bacteria (102,103).

The quiescent phenotype is actively present in chronic and recalcitrant infections surviving under antibiotic pressure . The slow-growing small-colony variant (SCV) subpopulation is another phenotype that complicates biofilm formation as well as treatment and diagnosis of biofilm-related disease. In combination, these different

phenotypes within microbial communities give rise to an extremely resilient community that can withstand many stressors and shield the resident bacteria from eradication.

Adaptive and phenotypic biofilm variations leads to difficulty in investigation and understanding the infection mechanisms . The interaction among various pathogenic species enhances horizontal gene transfer of clinically prevailing phenotypic resistance elements. An important characteristic of pluralism in biofilm composition is based upon the presence of nonbacterial elements.

Microbiological evaluation

According to microbiological reporting protocol, a micro organism in heavy growth from nasopharynx can be a colonizer or a pathogen based on the presence or absence of the clinical symptoms and signs.

Samples are inoculated onto primary media blood agar and chocolate agar. Incubation period of 24-48 hours is allowed and organisms are identified based on the standard operating protocol of the laboratory.

Individual organisms are identifies based on their characteristic colonies and confirmed using biochemical reactions as per laboratory protocols.

Biofilm ability for culture positive organisms

Biofilm screening assay is performed in the following manner. Briefly, 5 to 10 colonies from fresh overnight cultures were inoculated into a 10 ml Mueller-Hinton broth (MHB) containing 1% glucose and incubated at 37°C for 12-18 h. Following incubation, 0.05 Optical Density (OD) cells at 625 nm was prepared using a spectrophotometer (Shimadzu, Kyoto, Japan). Prepared cell suspension was inoculated in a 96-well plate and incubated at 37 °C for 24 hours. Biofilms were then washed with 200 µl of distilled water and stained with 200 µl 0.1% (w / v) crystal violet dye and incubated for 10 min at RT. After a water wash, plate was de-stained with 33% glacial acetic acid for 5 mins at RT. OD was recorded at 570 nm. The assay was performed in triplicates. Broth without cells was used as a negative control. The biofilm forming ability was classified as: $OD < ODc$ = poor biofilm producer; $ODc < OD \leq 2 \times ODc$ = weak biofilm producer; $2 \times ODc < OD < 4 \times ODc$ = moderate biofilm producer; and $OD \geq 4 \times ODc$ = strong biofilm producer.

Summary

Adenoid hypertrophy is a common problem seen in children between the age of 8-15 presenting to ENT OPD. They present with various symptoms such as nasal obstruction, nasal discharge, snoring , mouth breathing and OSA. It is also be associated with recurrent middle ear infections . The nasopharynx harbour various micro organisms which can be a commensal or pathogenic in nature. These microorganisms plays a vital role in the progression of acute infection and can lead to chronic inflammatory conditions causing recurrent ear infections

The better understanding of microbiological profile will help us in better management of symptoms arising due to adenoid hypertrophy. It can also contribute to recent trends in treatment of adenoid hypertrophy or adenoiditis with probiotics

However the exact profile of microorganisms seen in the nasopharynx of children with adenoid hypertrophy as compared to those with normal adenoids is not clearly studied in the past. So the study was planned to study the microbiological flora of the nasopharynx in children with and without adenoid hypertrophy . Furthur on ,to quantify the commensal and pathogenic bacteria in both the groups and to assess the biofilm forming capacity of these pathogenic group.

MATERIALS AND METHODS

This prospective observational study was conducted at the Department of Otorhinolaryngology, Christian Medical College, Vellore. Approval of the Institutional Review Board at Christian Medical College was obtained from IRB, Min. No. 12412 dated 02/12/2019.

Children less than 16 years of age presenting to ENT II OPD with symptomatic

adenoid enlargement were invited to participate in the study. Asymptomatic children less than 16 years of age without any nose, ear or throat complaints (for example – children undergoing hearing loss evaluation, neck swelling evaluation , recurrent upper airway infection not on antibiotics in the recent one month, children from Child health OPD and immunization clinic) were informed about the study and invited to participate as the control group

Study design

Prospective Observational study

Study Period

January 2020 to November 2021

Setting: The Study was conducted at the Department of ENT UNIT II and Department of Microbiology in Christian Medical College, Vellore.

Children were recruited from the ENT II OPD

Participants:

Children who presented with history of symptomatic adenoid enlargement were included in the study after taking an informed consent. Age matched children without any ear, nose or throat complaints were taken as control group and were evaluated as per clinical protocol

Inclusion criteria for cases - Children aged less than 16 years with history and clinical features of adenoid enlargement along with X ray of nasopharynx or Rigid Nasal Endoscopy (RNE) findings suggestive of symptomatic adenoid enlargement

Exclusion criteria for cases

- Caregivers not willing to participate

- Immunocompromised children
- Prior surgical procedure in upper airway
- Use of antibiotics or symptoms of URI within last 4 weeks
- Children with anomalies

Inclusion criteria for controls

- Children less than 16 years of age with no nose, ear or throat complaints (for e.g – children undergoing hearing loss evaluation, neck swelling evaluation , recurrent upper airway infection not on antibiotics in the recent 1 month, children from Child health OPD and immunization clinic .

Exclusion criteria for controls

- Caregivers not willing to participate
- Immunocompromised children
- Children with cervical anomalies
- Antibiotic use in the last 1 month

Methodology

Children who fulfilled the inclusion and exclusion criteria were invited to participate and were provided with an information sheet (Annexure). Those willing to participate were consented and then a detailed questionnaire was administered.

History was obtained from a reliable source. The questionnaire included three sections eliciting demographic details, clinical details and examination findings.

Modified Kuppusamy scale (Annexure) which uses a combination of education, income and occupation was used to grade the socioeconomic status of the study population as follows

Upper	26-29
Upper middle	16-25
Lower middle	11-15
Upper lower	6-10
Lower	<5

Figure 7: Socioeconomic grading

A complete ENT examination including otoscopy was carried out in the ENT OPD setting and findings were documented for cases and control.

As a part of routine clinical assessment, rigid nasal endoscopic examination or X ray soft tissue of nasopharynx lateral view was done to assess the size of the adenoid.

On X-ray Neck lateral view adenoid hypertrophy was graded based on the percentage of airway in nasopharynx as :

GRADE 1	<25%	
GRADE 2	26-50%	
GRADE 3	51-75%	
GRADE 4	>76%	

Figure 8: Grading of adenoid on an X ray

Rigid nasal endoscopic examination

It provides a three dimensional view of the nasopharynx and is more accurate in measuring the size of adenoids and degree of choanal obstruction. The grading is as follows

A - Adenoid size	C - Choanal obstruction	E - Eustachian tube
0 - No adenoids	0 - No obstruction	0 - Not abutting Eustachian tube
1 - 1% to 25%	1 - <50% obstruction	1 - Abutting Eustachian tube
2 - 26% to 50%	2 - >50% obstruction	
3 - 51% to 75%		
4 - 76% to 100%		

Figure 9: Adenoid grading on Rigid nasal endoscopy

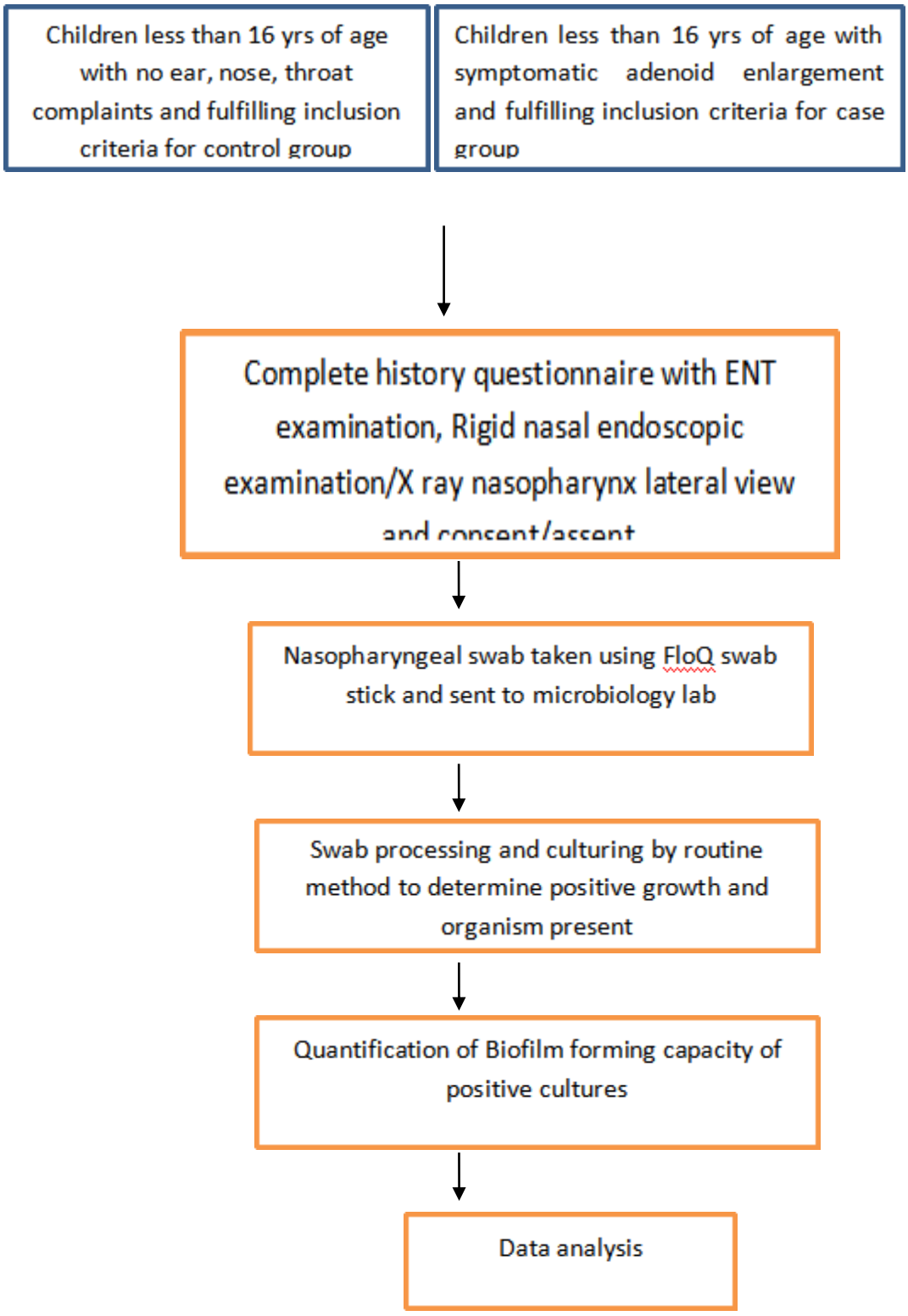

Figure 10: Workflow diagram for sample collection and processing

Nasopharyngeal Swab

A nasopharyngeal swab was taken for children in both study and control group.

FloQ nasal swab was used which has protruding nylon fibers that improve the recovery of target organisms from the sampled surface, and allow for the rapid dilution of collected material into the transport medium

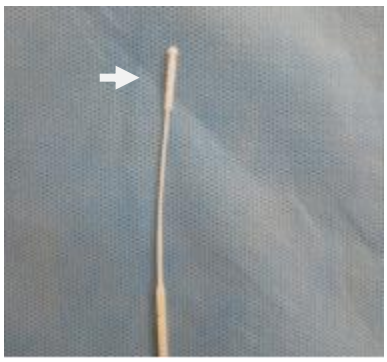

Figure11 : FloQ swab with nylon fibre tip (arrow)

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The process of sample collection is described below:

Hold the infant or young child's head securely. Tip their head backwards slightly and pass the swab directly backwards, parallel to the base of the nasopharyngeal passage. The

swab should move without resistance until reaching the nasopharynx, located about one-half to two-thirds the distance from the nostril to ear lobe

Collection and Transport

Figure 12: FloQ swab with STGG medium

The nasopharyngeal (Np) samples were collected in STGG medium (Skim milk Tryptone Glucose Glycerol), which is the recommended nasopharyngeal swab transport medium for detection of *S. pneumoniae* (104). The nasopharyngeal swabs were collected from one of the nostrils using nylon flocked swabs from HI Media as per the CDC guidelines . Immediately following sample collection, the NP swab is aseptically placed into the vials containing 1ml STGG medium, inserting it to the bottom of the vial, raising it slightly and cutting off the shaft with sterile scissors (to enable lid closure), leaving the swab in the STGG media. The closed tube is then placed in a cool box or on wet ice (4^0 C) and transported to the laboratory within 8 h.

Once in the laboratory, the specimen vial is vortexed at high speed for 10–20 s to disperse organisms from the swab tip and stored in freezer until processed (-70°C).

Processing

Reaching the Microbiological laboratory,

- Inoculate NP swabs on to blood agar (BA), chocolate agar (CA) and mannitol salt agar (MSA). Incubate all cultures at 37° C over night. Incubate BA and CA in CO_2 atmosphere.
- Sheep blood agar is recommended for the isolation of *S. pneumoniae* as colony size morphology and even minimal haemolysis is better appreciated on this. Inoculate CA for all specimens

Culture Follow-up:

After the overnight incubation the culture growth is read followed by Gram stain and special tests to identify the pathogen. Report all the pathogens with heavy or moderate growth.

Antimicrobial susceptibility testing is done for all pathogens reported.

Identification of the Pathogens

S.Pneumonia, *H. influenzae*, *S. aureus*, *K. pneumoniae* and Non fermenting gram-negative bacilli (NFGNBs) are the main pathogen species looked for in the nasopharyngeal swabs.

Following the Gram stain morphology, for Gram positive cocci arranged in clusters, specialized tests can be used to identify *Staphylococcus aureus.*

The tests are catalase test, coagulase test, tube coagulase, clumping factor test, bound coagulase, and susceptibility to Furozolidone.

Blood agar plates with alpha hemolytic colonies were sub-cultured and tested for the confirmation of *S. pneumoniae* using CDC protocol. The *S. pneumoniae* isolates (Figure 13) confirmed using bile solubility test positive (bile soluble) and optochin (5μg disk concentration) susceptible were further processed for serotyping .

Serotyping of *S. pneumoniae* was done by Quellung method using pneumococcal antisera from Staten's Serum Institute, Denmark and the conventional sequential multiplex PCR (105) .

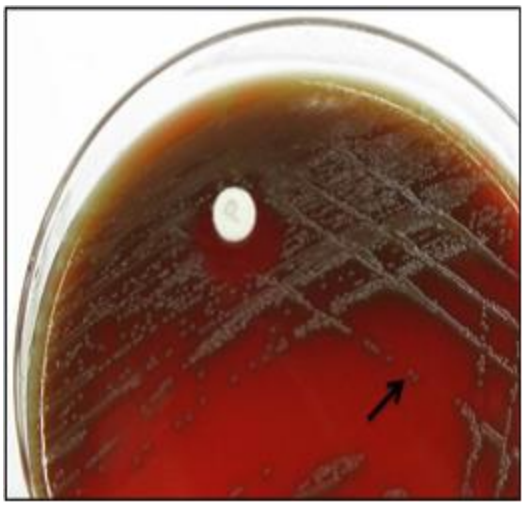

Figure 13 : *S. pneumoniae* colonies with a surrounding green zone of alpha hemolysis (blackarrow) on a BAP

H.influenzae are small, pleomorphic, gram-negative bacilli or coccobacilli with random arrangements. *H. influenzae* is a fastidious organism which grows best at 35-37°C with ~5%CO2 (or in a candle-jar) and requires hemin (X factor) and nicotinamide-adenine-dinucleotide (NAD, also known as V factor) for growth. *H. influenzae* appear as large, round, smooth, convex, colorless to-grey, opaque colonies on a CAP. The tests such as Kovac's oxidase test and determining the necessity of hemin and NAD as growth requirements are recommended to confirm the identity of cultures that morphologically appear to be *H. influenzae* (Figure 14).

H. If the oxidase test is positive, hemin and NAD growth factor requirement testing should be performed (Table 1). If the growth factor requirement test indicates that the isolate may be *H. influenzae*, serological tests to identify the serotype should be performed

Figure 14: Translucent colonies of *H. influenzae* on a chocolate agar plate

Table 1. Identification of *Haemophilus* spp. by their growth requirements for hemin (X factor) and NAD (V factor) and β-hemolysis on horse blood agar

V = variable, + = positive, - = negative

Species	*Growth requirements*		**Hemolysis on 5% horse blood or rabbit blood**
	X	**V**	
H. influenzae	+	+	-
H. parainfluenzae	-	+	-
H. haemolyticus	+	+	+
H. parahaemolyticus	-	+	+
H. ducreyi	+	-	V

Following the Gram stain morphology, the colonies showing gram negative bacilli are first screened using Oxidase test. *Klebsiella pneumoniae* belongs to family enterobacteriaceae are oxidase negative. Pseudomonas and Acinetobacter species are non-fermenting gram-negative bacilli.

Further preliminary screening media like Mannitol motility medium, Triple sugar iron agar medium, Peptone water and Citrate are used to differentiate the enterobacterial species (106)

Biofilm ability for culture positive organisms

Biofilm screening assay was performed in the following manner. Briefly, 5 to 10 colonies from fresh overnight cultures were inoculated into a 10 ml Mueller-Hinton broth (MHB) containing 1% glucose and incubated at 37°C for 12-18 h. Following incubation, 0.05 Optical Density(OD) cells at 625 nm was prepared using a spectrophotometer (Shimadzu, Kyoto, Japan). Prepared cell suspension was inoculated in a 96-well plate and incubated at 37 °C for 24 hours. Biofilms were then washed with 200 µl of distilled water and stained with 200 µl 0.1% (w / v) crystal violet dye and incubated for 10 min at RT. After a water wash, plate was de-stained with 33% glacial acetic acid for 5 mins at RT. Optical

density (OD) was recorded at 570 nm. The assay was performed in triplicates. Broth without cells was used as a negative control.

The biofilm forming ability was classified as:

$OD < ODc$ = poor biofilm producer

$ODc < OD \leq 2 \times ODc$ = weak biofilm producer

$2 \times ODc < OD < 4 \times ODc$ = moderate biofilm producer

$OD \geq 4 \times ODc$ = strong biofilm producer

Sample size calculation

It's a case control study to compare microbiological profile on nasopharynx in children with symptomatic adenoids and normal children, a sample size of 90 with 60 children in case group and 30 children in control group was decided. Since no previous studies are available that compares the population mentioned above , we have taken a similar study done in Department of ENT 2 as our reference. The study- " Comparison of microbiotia of throat in children with recurrent tonsillitis versus asymptomatic children- a pilot study looked at 80 patients, 40 with recurrent tonsillitis(study arm) and 40 asymptommatic patients(control arm)

Statistical analysis

All study variables will be summarized using descriptive statistical methods. Continuous outcome variables will be compared between the study group (case/control) using independent two sample t-test. Chi- square test will be used for categorical variables. Absolute difference in the means and proportions of outcome variables will be reported along with 95% confidence.

RESULTS

There were a total of 90 patients included in the study “To identify and compare microbiological profile of nasopharynx in children with features of symptomatic adenoid enlargement versus asymptomatic normal children”

The study group had 60 patients and control group had 30.

The results were analyzed in the following subgroups

Demographic profile

Clinical profile

Microbiological profile

A) DEMOGRAPHIC PROFILE

1) Age

The children recruited in study and control group were in the age group 3-15 years. The age groups were subdivided as less than 7 years, 7-10 years and 11-15 years.

Majority of children presenting with symptomatic adenoid enlargement were under 7 years of age.

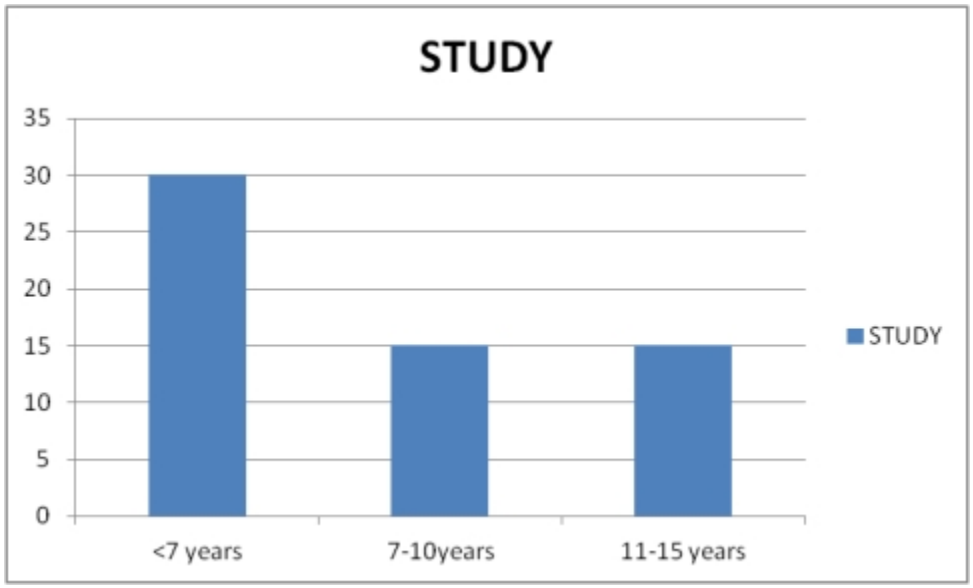

Figure 15: Age distribution in study group with number of children represented on Y axis

In the control group the age group with most number of children was also found to be less than 7 years.

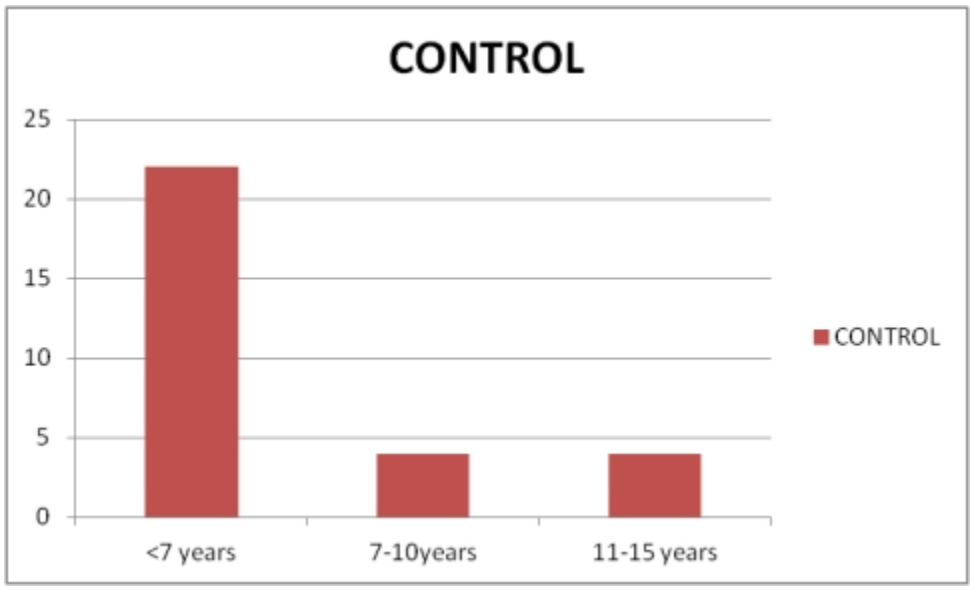

Figure 16: Age distribution in control group with number of children represented on Y axis

2) Sex distribution

In both study and control group, number of boys were more than number of girls.

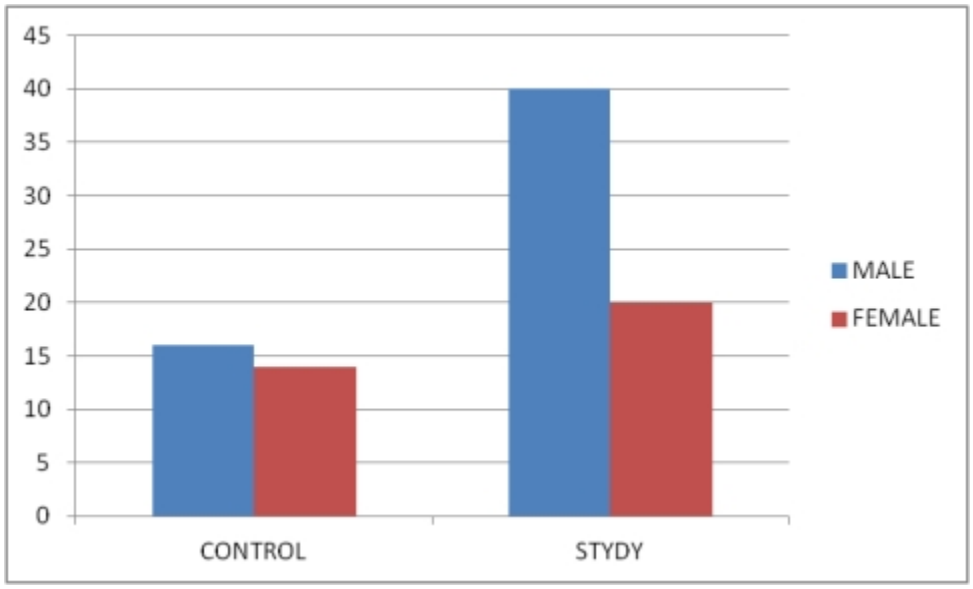

Figure 17: Sex distribution in study and control group . Number of children represented in Y axis

3)Socio-economic profile

Modified Kuppuswamy scale was used to assess the socioeconomic status. SES based on the score were grouped as follows: <5- Lower, 5-10 Upper lower, 11-15 Lower middle, 16-25 Upper middle, 26-29 Upper

In our study most of the children in both study and control group were from upper middle (43%) socioeconomic group

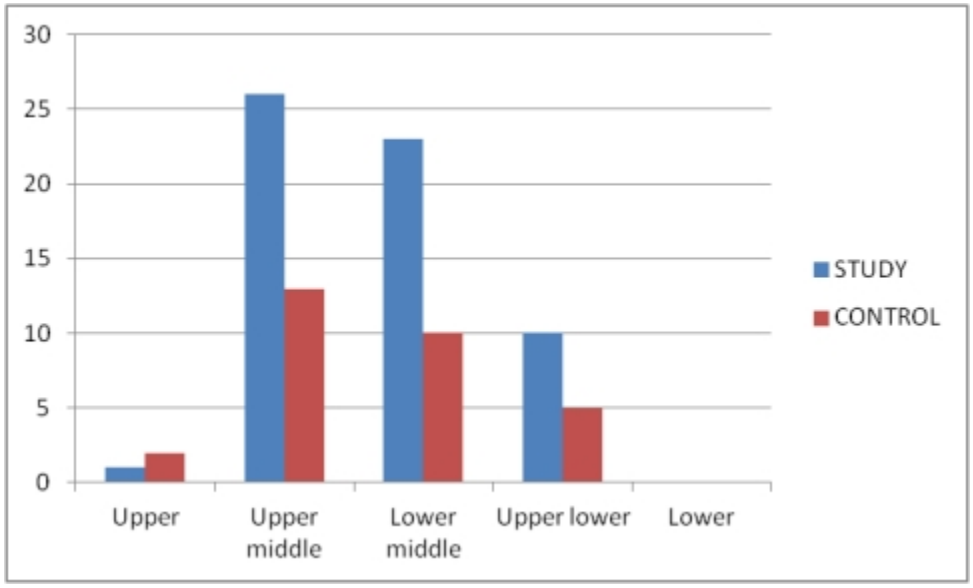

Figure 18: SES of children in study and control group with numbers represented on Y axis

4) Geographic distribution

Most children in our study were from Tamil Nadu followed by West Bengal

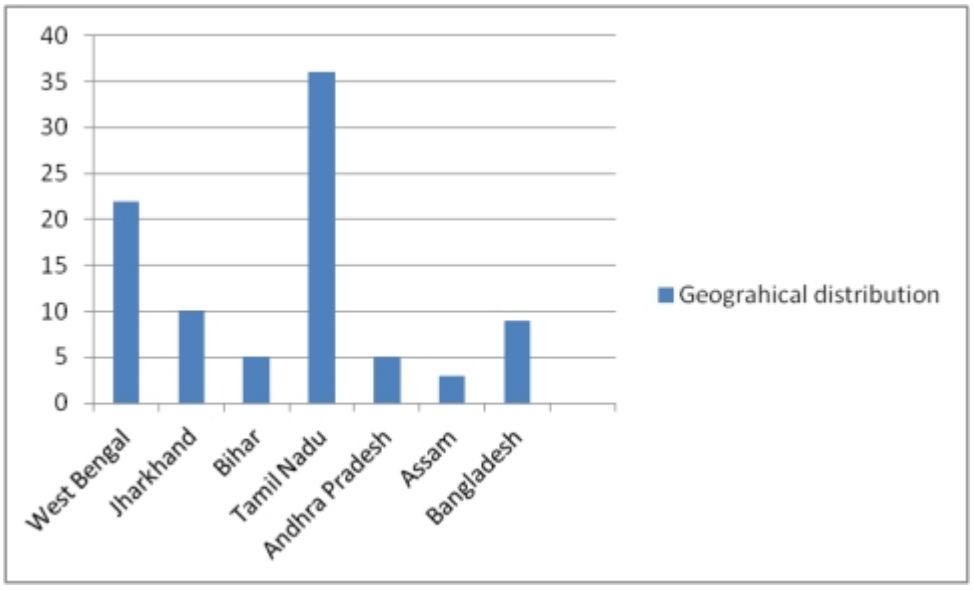

Figure 19: Geographical distribution of all patients with number on Y axis

CLINICAL PROFILE

1) Presenting Symptoms in the study group

The study group consisted of children who presented to OPD with symptoms suggestive of adenoid enlargement. Most children had multiple symptoms. The commonest presenting complain was found to be nasal block (50%), followed by nasal discharge and snoring.

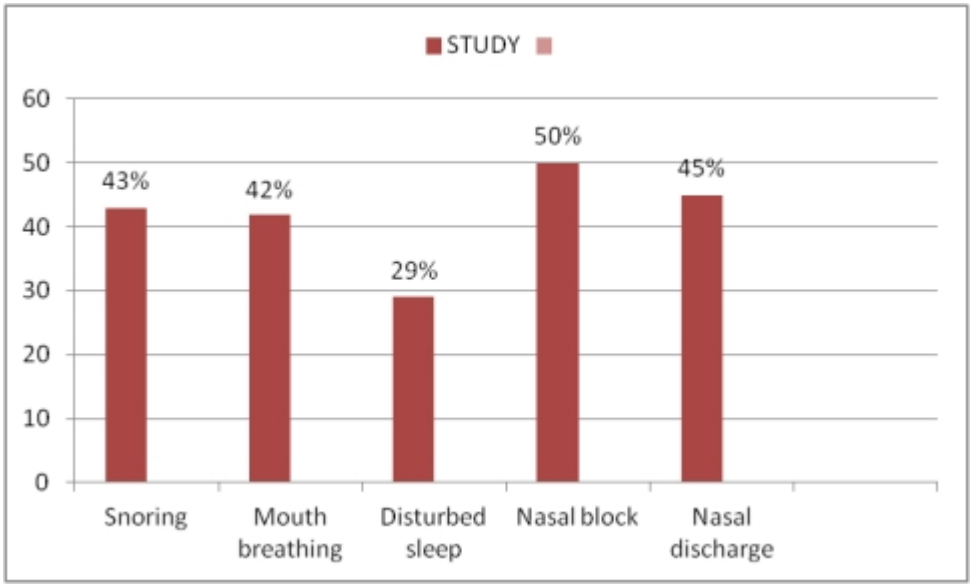

Figure 20: Presenting symptoms in children with adenoid hypertrophy.

Y axis representing number of children who had that symptom

2) Associated symptoms seen with adenoid enlargement

Children in the study group who primarily had symptoms suggestive of adenoid hypertrophy also gave history of symptoms related to nose and throat. The commonest associated symptom was of recurrent sore throat

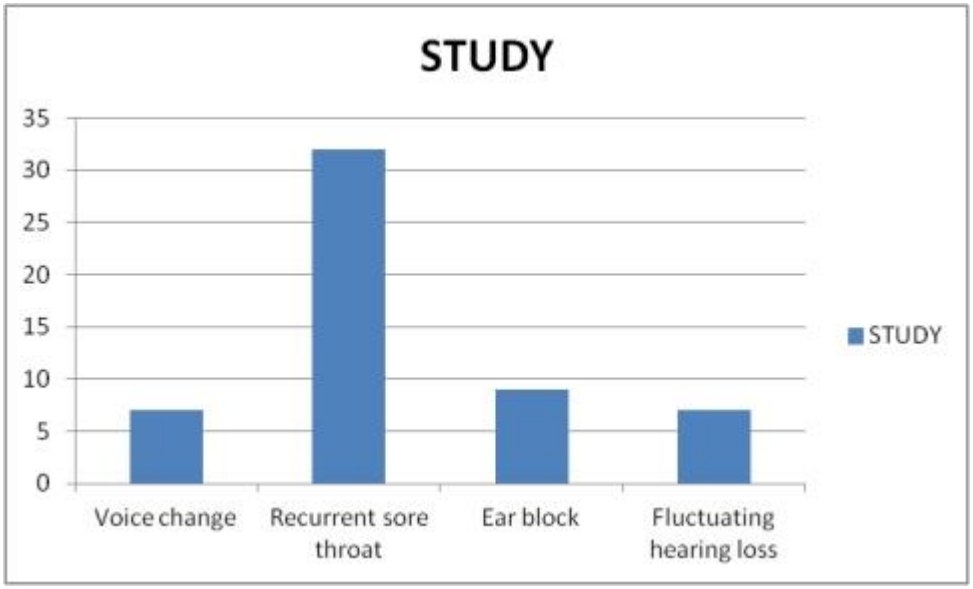

Figure 21: Additional symptoms in study group with number of children represented on Y axis

3) Immunization status

In our study we found that out of 90 children, 76 were adequately immunized as per their age. Caretakers of 14 children did not know the immunization status of the child. The vaccines that we included were BCG, OPV, DPT, MMR, HiB, Measles and Pneumococcal.

4)Clinical findings in children with adenoid enlargement

Children in the study group were found to have following findings in nose and ear. The commonest finding was that of pale looking nasal mucosa along with discharge seen in the nasal cavity

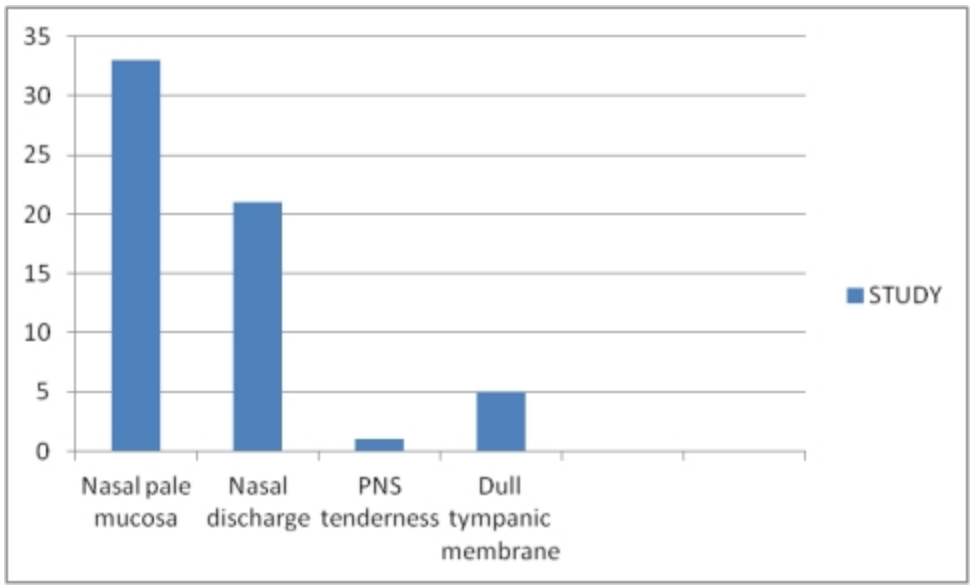

Figure 22: Nose and ear findings on clinical examination of study group. Numbers are represented on Y axis

5)Tonsillar hypertrophy in children with adenoid hypertrophy

On examination of the children in the study group, 68% of them had tonsillar enlargement and 32% did not.

6) Grade of adenoid hypertrophy

The grading was done based on Cohen and Konak's system for X ray soft tissue nasopharynx lateral view and A.C. E grading system for rigid endoscopy.

The commonest finding was grade 3 adenoid hypertrophy which was present in 60% of individuals

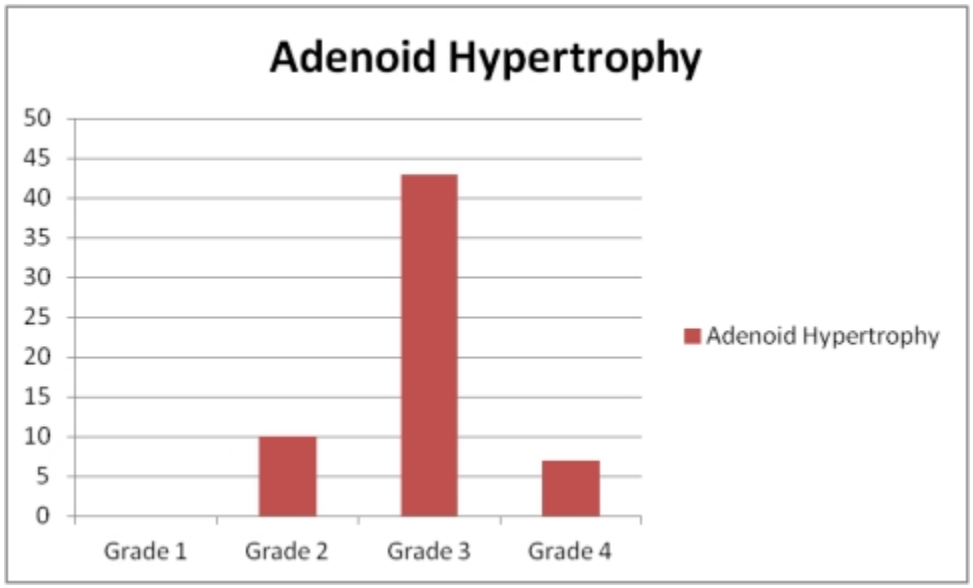

Figure 23: Grades of adenoid hypertrophy with number of children represented on Y axis

MICROBIOLOGICAL PROFILE

The microorganisms cultured were classified as normal flora and pathogenic group.

1)Microbiological profile in children with symptomatic adenoid enlargement

In the study group, majority of the cases had normal flora as the growth and the commonest pathogenic bacteria was found to be *Staphylococcus aureus and Streptococcus pneumonia.*

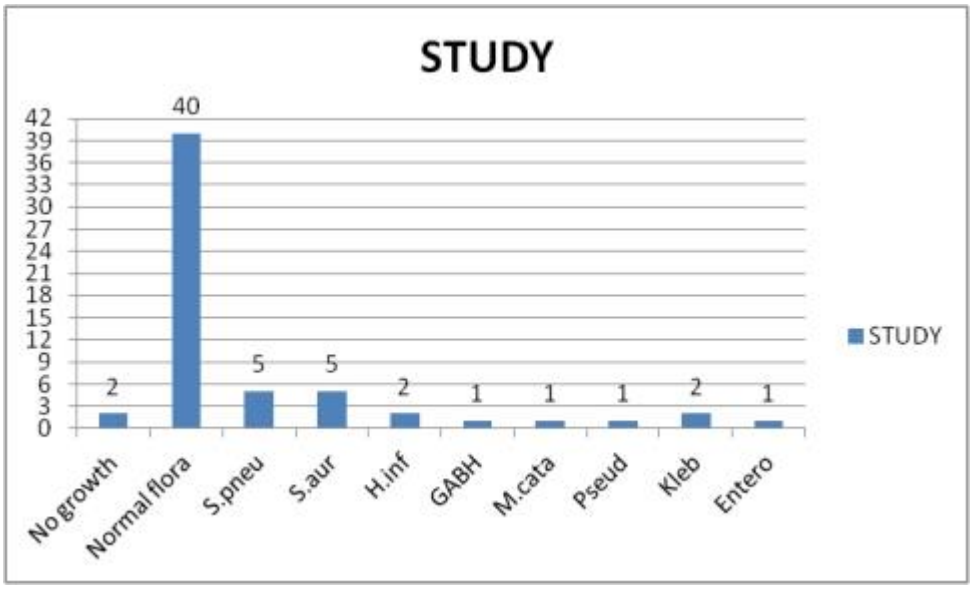

Figure 24: Distribution of micro organisms in study group and number of children represented on Y axis

S.pneu- *Streptococcus pneumonia*, S.aur- *Staphylococcus aureus*, H.inf- *Haemophilus influenza*, GABH- *group A beta hemolytic streptococci*, M.cata- *Moraxella catarrhalis*, Pseud- *Pseudomonas*, Kleb- *Klebsiella*, Entero- *Enterobacter*

2) Microbiological profile in control group

Streptococcus pneumonia was found to be common pathogenic bacteria after commensals

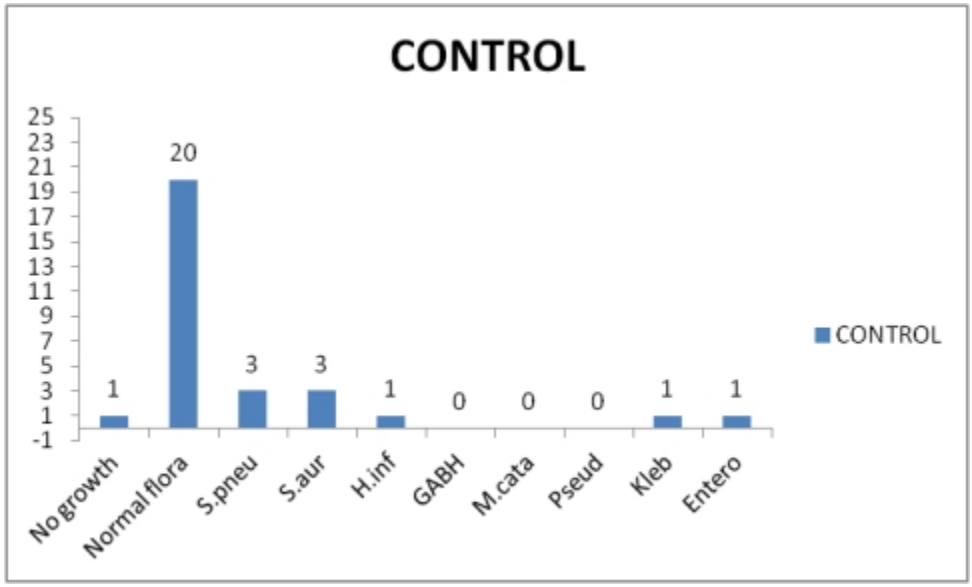

Figure 25: Micro organisms in control group and the number of children on Y axis

S.pneu- *Streptococcus pneumonia*, S.aur- *Staphylococcus aureus*, H.inf- *Haemophilus influenza*, GABH- *group A beta hemolytic streptococci*, M.cata- *Moraxella catarrhalis*, Pseu- *Pseudomonas*, Kleb- *Klebsiella*, Entero- *Enterobacter*

3) Comparison of the microflora.

In the study group and the control group, commensal were the commonest growing organisms.

Also, *Staphylococcus aureus* and *Streptococcus pneumonia* were the common pathogenic bacteria in both the groups.

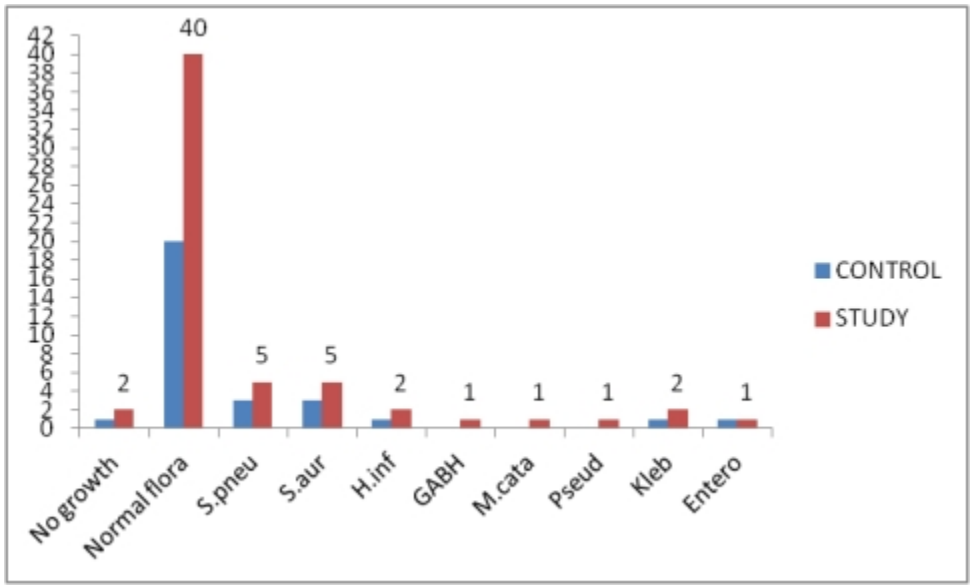

Figure 26: Comparison of microflora in study and control group with number of children represented on Y axis

S.pneum- *Streptococcus pneumonia*, S.aur- *Staphylococcus aureus*, H.inf- *Haemophilus influenza*, GABH- *group A beta hemolytic streptococci*, M.cata- *Moraxella catarrhalis*, Pseu- *Pseudomonas*, Kleb-

4) Age dependent trend in microflora

Commensals were more common in age less than 7 years as compared to other ago groups.

More pathogenic bacteria in age 7-15 years

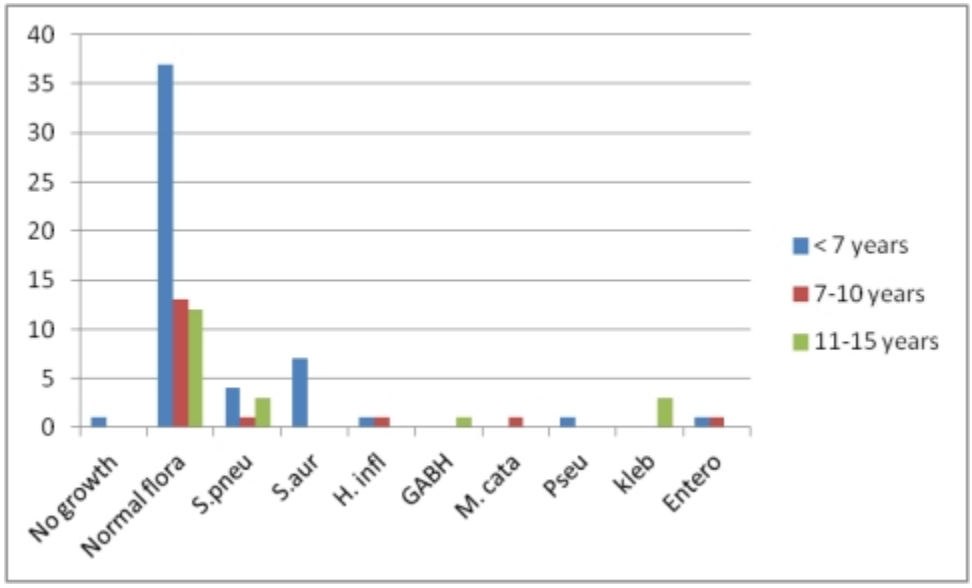

Figure 27: Microorganism as seen in different age groups with number on Y axis

S.pneum- *Streptococcus pneumonia*, S.aur- *Staphylococcus aureus*, H.inf- *Haemophilus influenza*, GABH- *group A beta hemolytic streptococci*, M.cata- *Moraxella catarrhalis*, Pseu- *Pseudomonas*, Kleb- *Klebsiella*, Entero- *Enterobacter*

5) Capacity to form Biofilm in culture positive organisms

In both study and control group it was found that pathogenic organism had a great capacity to form biofilm.

In Study group out of 16 positives; 11 organisms had the capacity to form moderate to heavy biofilm (68%)

In Control group out of 9 positive; 5 had the capacity to form moderate to heavy biofilm (55%)

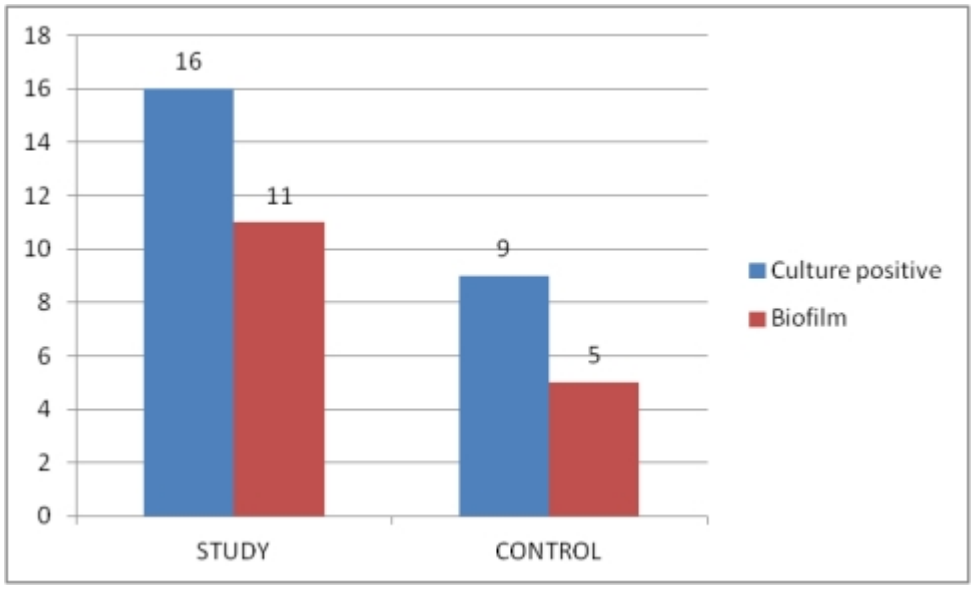

Figure 28: Capacity of pathogenic microorganism to form biofilm in study and control group with number of children on Y axis

6) Capacity of organisms to form biofilm

Staphylococcus aureus was associated with maximum capacity to form biofilm

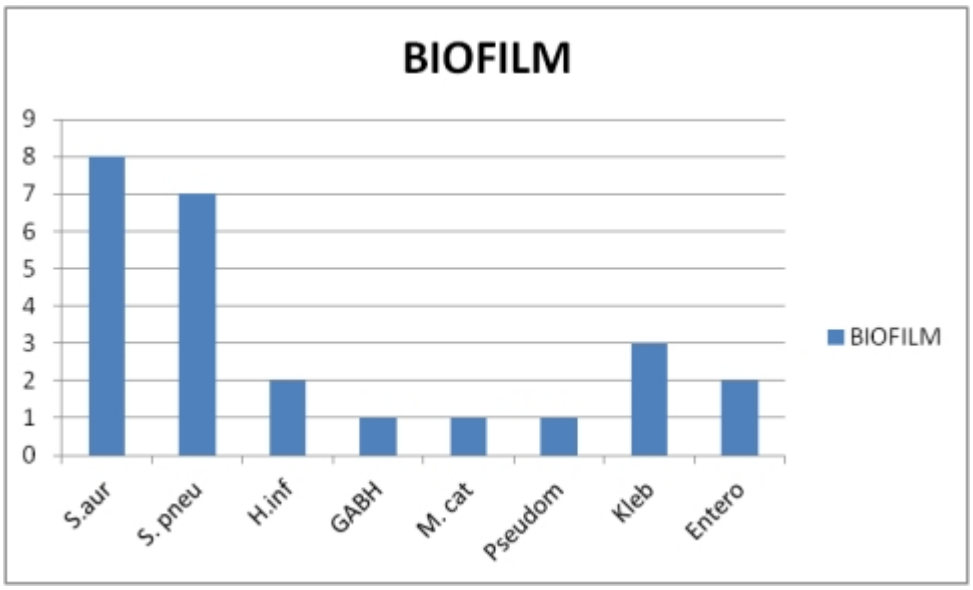

Figure 29: Capacity to form biofilm with number on Y axis

S.pneum- *Streptococcus pneumonia*, S.aur- *Staphylococcus aureus*, H.inf- *Haemophilus influenza*, GABH- *group A beta hemolytic streptococci*, M.cata- *Moraxella catarrhalis*, Pseu- *Pseudomonas*, Kleb- *Klebsiella*, Entero- *Enterobacter*

7) Strength of the organisms to form biofilm

Staphylococcus aureus showed the presence of heavy growth in isolates that formed biofilm.

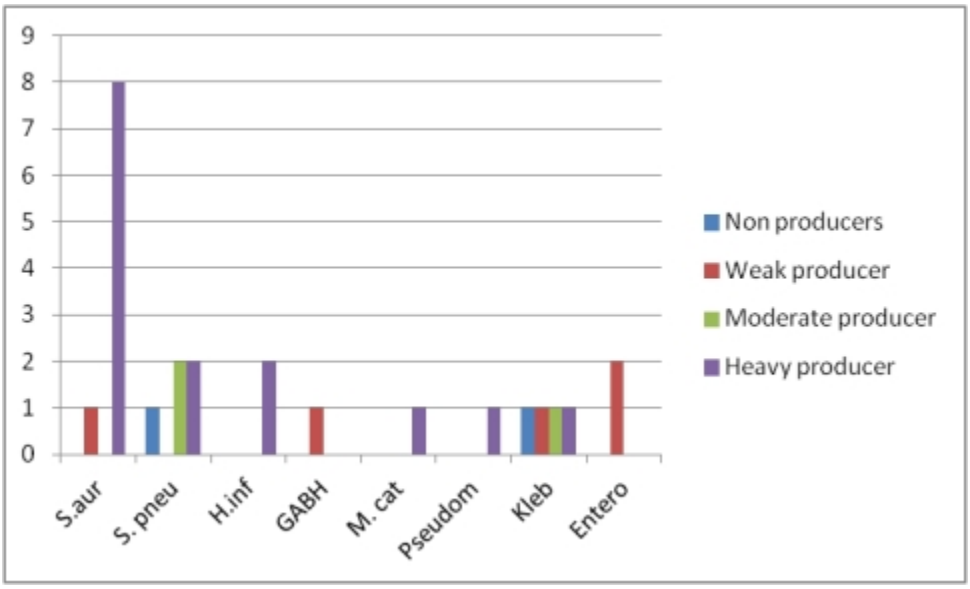

Figure 30: Growth of microorganisms represented by different color with number on Y axis

S.pneum- *Streptococcus pneumonia*, S.aur- *Staphylococcus aureus*, H.inf- *Haemophilus influenza*, GABH- *group A beta hemolytic streptococci*, M.cata- *Moraxella catarrhalis*, Pseu- *Pseudomonas*, Kleb- *Klebsiella*, Entero- *Enterobacter*

8) Overcrowding and culture positivity

64% of the children who had positive pathogenic bacterial growth on swab were living in houses with more than 4 people

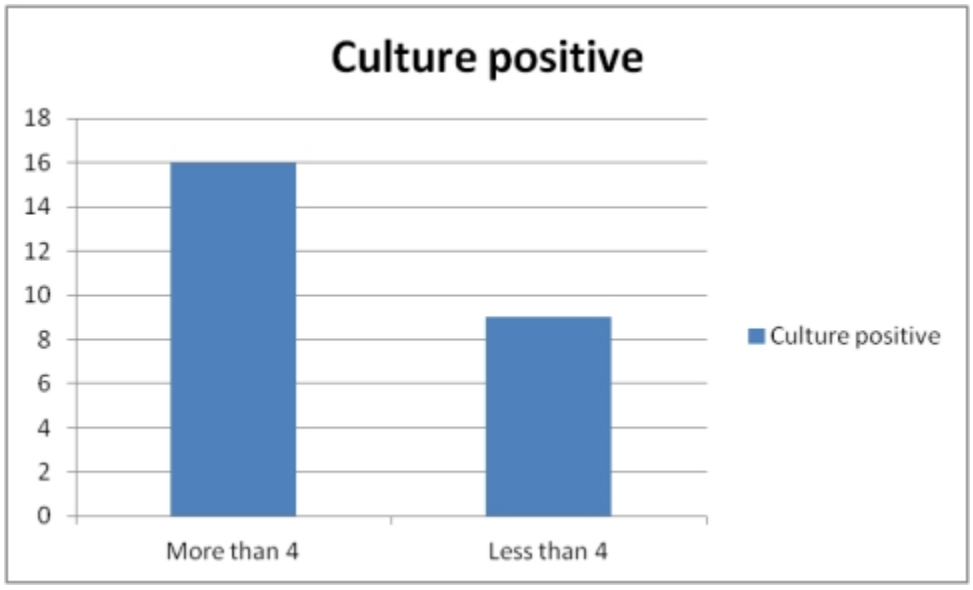

Figure 31: Association between pathogenic culture positives and number of people in the house with Y axis representing the number of children

9) Semi quantification of commensals

The samples that had commensals as growth were semi quantified and we found that *Coagulase negative Staphylococcus* (CONS) was the commonest commensal. Some children had more than one commensal growth.

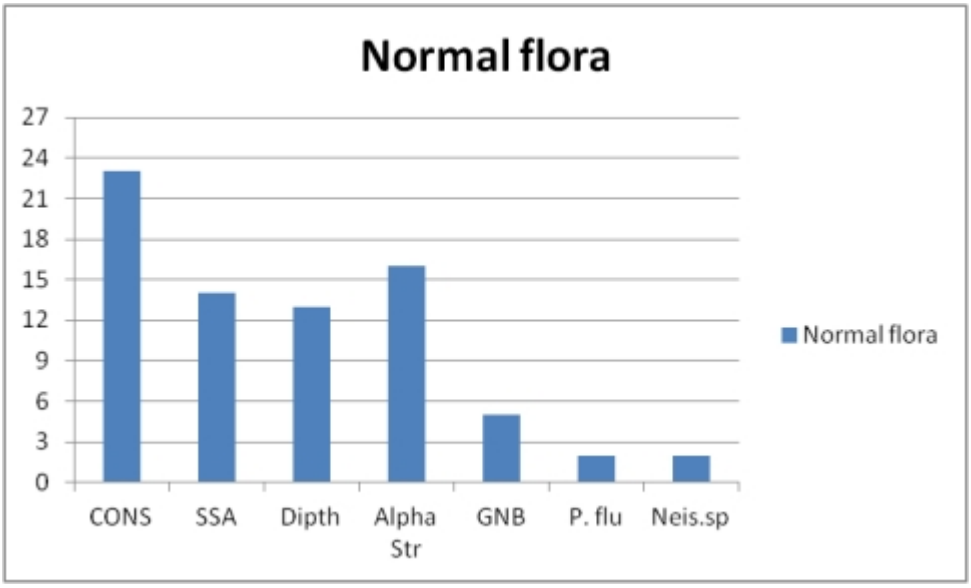

Figure 32: Semi quantification of various commensal and number of children on Y axis

CONS- *Coagulase negative staphylococcus*, SSA- *Scanty Staphylococcus aureus* Dipth- *Diptheroids*, Alpha str- *Alpha hemolytic Streptococcus*, GNB- *gram negative bacilli*, P.flu- *Pseudomonas fluorescens*, Neis.sp- *Neisseria species*

10)Comparison of commensal in Study and control group

In study group *Coagulase negative staphylococcus* and *alpha hemolytic streptococci* were the common commensal and in control group *coagulase negative staphylococcus* was the commonest commensal.

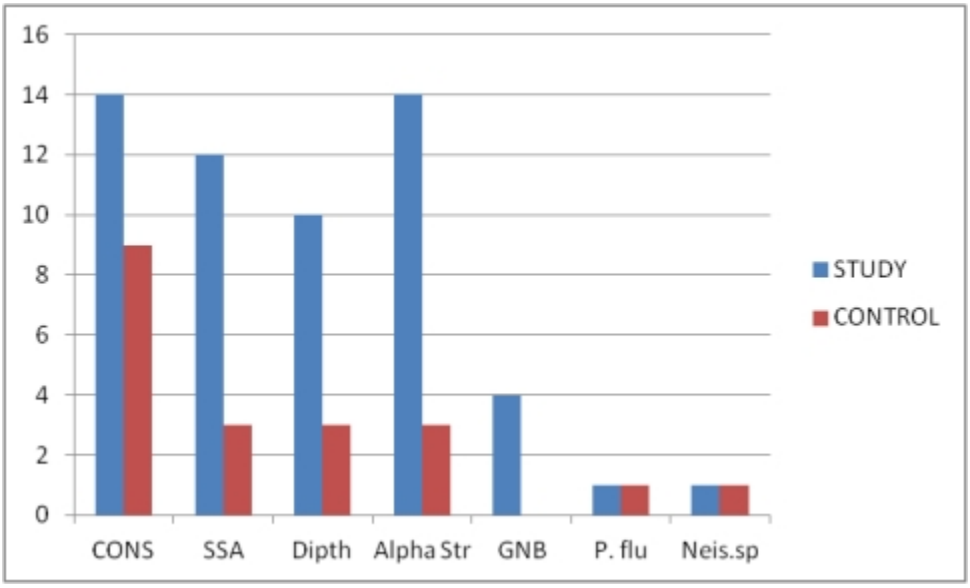

Figure 33: Comparison of commensal in control and study group with number of children on Y axis

CONS- *Coagulase negative staphylococcus*, SSA- *Scanty Staphylococcus aureus* Dipth- *Diptheroids*, Alpha str- *Alpha hemolytic Streptococcus*, GNB- *gram negative bacilli*, P.flu- *Pseudomonas fluorescens*, Neis.sp- *Neisseria species*

DISCUSSION

In this study 60 children with history of symptomatic adenoid enlargement and 30 children without any ear, nose or throat related complaints were included. We studied and compared the microbiotia in nasopharynx of both the groups.

Children from 3 to 16 years of age were included in the study as per the inclusion and exclusion criteria for both study and control group. Usually 3-8 years is the age when adenoid enlargement takes place and is physiologically normal. But, it can result in airway obstruction and sleep disorders. It can also cause Eustachian tube dysfunction, leading to serous otitis media and if not treated adequately can result into chronic suppurative otitis media.

Adenoids situated in the nasopharynx, is a potential site for growth of microbes and houses many aerobic and anaerobic microbes which can be a part of normal flora or could be pathogenic in nature. This study was conducted to get a better understanding of the microflora of the adenoids.

In this study majority of children who presented with symptoms of adenoid hypertrophy were less than seven years of age and this is favoring the literature according to which 3-8 years is the age when growth of adenoids is maximum.

The maximum children in control group were also less than 7 years, and this could be because our control group included children with congenital hearing loss, congenital neck masses, respiratory disorders which presents early in life.

The number of boys were more in both study and control group. The available studies however does not suggest any predilection towards a particular sex.

The geographical areas from where the control and study population belonged to were varied. Most children were from the state of Tamil Nadu, however there were a significant number of children from North Eastern states, the maximum being from West Bengal, which corresponds to patient distribution pattern of the ENT department where study was conducted.

We observed that most children in study group as well as control group belonged to upper middle and lower middle socioeconomic class. In general, low socioeconomic status is considered an important factor in increasing the chances of infection.

Few other observations made in the study were that 95.5 % children were vaccinated adequately as per their age and none of them had a history of previous surgery or use of antibiotics in the past four weeks.

Children in study group presented with multiple symptoms suggestive of adenoid enlargement. The commonest symptom was that of nasal block followed by nasal discharge. Hypertrophied adenoids causes obstruction at the level of choana resulting in nasal block. Nasal discharge could be due to associated allergic rhinitis, and from literature we known that children who have history of allergic rhinitis are more prone to developing adenoid hypertrophy (1). They also gave history of snoring and mouth breathing.

32% of the children in study group also gave history of recurrent sore throat and on examination were found to have tonsillar hypertrophy. The growth of lymphoid tissue in the waldeyers ring secondary to infections can be well observed here.

On clinical examination the commonest finding was that of pale looking nasal mucosa probable secondary to allergic rhinitis. The adenoid size was graded using standardized grading system and it was observed that grade 3 of adenoid enlargement was the commonest in the study group.

The study conducted by Brook and Shah observed that the commonest gram positive pathogenic bacteria were Group A beta and alpha hemolytic streptococci and *S.aureus*(2). Khalid et.al and Rajeshwari et.al had similar finding where they found that the commonest pathogenic gram positive bacteria were *S.aureus, S. pneumonia, Enterococcus* and *Group A beta hemolytic streptococci* and commensals were *S.pyogenes, S.epidermidis* and *S.viridans*. The gram negative pathogenic bacteria were *Pseudomonas, E.coli and Klebsiella* species whereas the commensals were *H.influenza* and *M.catarrhalis.*

In our study also we noted that in the study group that consisted of 60 children with symptoms of adenoid hypertrophy, most children had normal flora as the primary growth. In the potentially pathogenic bacteria group, it was observed that *Staphylococcus aureus* and *Streptococcus pneumonia* were the commonest gram positive aerobes, followed by *Group A beta hemolytic streptococcus and Enterococcus*. The commonest gram negative aerobes were *Klebsiella sp*, followed by *Pseudomonas.*

In the control group also we noted that the normal flora was the predominant growth and *S.aureus* and *S.pneumonia* were the main gram positive pathogenic bacteria followed by *Enterococcus*. The only gram negative aerobic bacteria cultured was *Klebsiella*.

The study by Rajeshwari et.al concluded that the pathogenic organism on the surface were similar to that of core tissue in adenoid, but the surface had more commensals as compared to the core in children with adenoid hypertrophy(3).

We made a similar observation in our study where we found that in both study and control group, commensals were more in number as the swab was taken from the adenoid surface. Another reason that can justify this is in our study the colonization was not quantified since none of the children had acute infection or history of surgery in the past and therefore , some pathogenic bacteria were also considered colonists and not infectious.

We also found that pathogenic organisms were found to be more in study group as compared to the control group.

Literature suggest that early childhood is associated with polymicrobial colonization with commensals being the predominant microbes and as age advances the commensals gets replaced by pathogenic bacteria due to repeated infections and use of antibiotics. In our study we found that children less than 7 years of age had mainly commensals growth and few had pathogenic bacteria lilke *S.aureus* and *S.pneumonia*. In age group 7-10 and 11-15 years there were lesser commensals comparatively and more pathogenic bacteria.

Children with history of recurrent adenoidal disease who do not respond to antibiotic therapy are usually candidates for surgery. It has been suggested that the ineffectiveness of antibiotic therapy in children with chronic adenoiditis is related to nasopharyngeal

bacterial biofilm and it results into chronic nasopharyngeal inflammation due to chronic adenoiditis which is possibly associated with chronic or recurrent middle ear disease.

The formation of biofilm on the surface of adenoids has been well established (4,5) . In our study we found that in the study group 68 % of pathogenic bacteria had moderate to strong capacity to form biofilm whereas in control group 55% of pathogenic bacteria were forming biofilm. It was further noted that organism associated with maximum capacity to form biofilm was *Staphylococcus aureus* followed by *Streptococcus pneumonia*.

Semi quantification of biofilm growth revealed that in majority of samples *S.aureus* was producing heavy growth , followed by *S.pneumonia* and *H.influenza*.

Previous studies where biofilm formation in nasopharynx has been studied shows that the organism mostly responsible for biofilm formation are *S.aureus, S.pneumonia and H.influenza* and this is similar to what we observed in our study as well(6).

We also semi quantified the commensal present in study and control group. Some cultures where the growth of pathogenic bacteria were insignificant, were considered as commensal due to their inability to cause infection. In study group we found that *Coagulase negative staphylococcus(* CONS) was the commonest commensal followed by *Alpha hemolytic streptococci*. In control group also CONS was found in majority of the samples.

Limitations

Our study had few limitations. The sample size was small and therefore the result might not be representative of the entire pediatric population.

The study took place during the COVID pandemic and there is a possibility that many children were given antibiotics as prophylaxis, that can alter the findings of our study.

Most of our samples were collected during the month of September to November 2021(due to lockdown) and could have been altered as during this time most children have URI due to cold weather.

CONCLUSION

The knowledge of microbiological profile of nasopharynx in children with adenoid enlargement is important as surface colonization of adenoid and associated biofilm formation could function as a reservoir for upper respiratory tract, ear and sinonasal infections. This study was done to study and compare the microbiotia of nasopharynx in children with symptomatic adenoid enlargement and asymptomatic children. It was observed that younger age group children (< 7 yrs) had more commensals and as age increased the proportion of the pathogenic bacteria increased. Pathogenic bacteria in both the groups were similar, but were found in greater number in study group. The capacity to form biofilm was maximum in symptomatic group and most pathogenic bacteria had heavy growth in the biofilm.

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ANNEXURE

CHRISTIAN MEDICAL COLLEGE, VELLORE

DEPARTMENT OF ENT

Protocol No:

Information Sheet

Name of participant:

Your child is invited to take part in this study. The information sheet will help you to understand the details of this study and to help you decide whether or not to take part in the study. Please feel free to ask if you have any doubts, I am happy to explain.

What is the study about?

The surface of the adenoids (the lymphoid tissue similar to tonsil, seen behind the nasal passage) has many folds. Its surface get colonised by many bacteria from the birth of the child, which includes the ones which causes disease (pathogenic bacteria) and the ones with protective function (commensal Bacteria). The interaction between these two types of bacteria will also decide whether the child has more chance for infection or more

protection from infection.. due to multiple reasons the adenoids can be enlarged in some children. This study is to understand the bacteria on the surface of the adenoids in children with adenoid enlargement versus normal adenoids by taking a swab from behind the nose. This swab will be analysed in the microbiology lab to understand the colonisation by bacteria

If you take part, what will you have to do?

If your child take part in the study, he/she will have to undergo a a simple nasopharyngeal swab test by passing a soft this swab deep into the nose , rotating it and taking it out. The swab will then be send to our microbiology lab for analysis.

Are there any risks for you if you take part in the study?

There is no major risk to the participant in the study The nasopharyngeal swab is a thin soft swab and the procedure takes two to three seconds and is usually well tolerated. Occasionally it can cause mild discomfort with minimal bleeding. In case if there is minimal bleeding, putting few nasal drops and pinching of the nose will settle the problem

Do you have to pay?

If your child has enlarged adenoids, you will have to pay only for the tests that are required for the routine treatment.

What are the benefits to you if you take part in the study?

If you participate in the study,

1. You will get a better understanding of the organisms colonizing the nasopharynx of your child
2. This knowledge will help us to make a better decision on the kind of treatment we would like to offr you if needed..

What are the possible benefits to other people?

This new knowledge will help us to understand the bacterial colonisation on the surface of the adenoids and their interaction better in children who has adenoid enlargement and normal adenoids. This information will contribute to develop newer ways of treatment for bacterial colonisation.

Can you decide not to participate?

Your child's participation in this study is entirely voluntary, and you are also free to decide to withdraw permission to participate in this study. If you do so, this will not affect your child's usual treatment at this hospital in any way. Your doctor will still take care of your child, and you will not lose any benefits to which you are entitled.

Will your personal details be kept confidential?

The results of this study may be published in a medical journal, but your child will not be identified by name in any publication or presentation of results. However, medical notes may be reviewed by people associated with the study, without your additional permission, should you decide to participate in this study.

If you have any further questions, you may contact

Dr.Joyna Singh

Department of ENT 2 & Pediatric ENT

CMC Vellore,

Mobile: 9629270768

Office: 04162282798

Informed Consent form to participate in a research study

1. **Study Title:** To identify and compare microbiological profile of nasopharynx in children with features of symptomatic adenoid enlargement versus asymptomatic normal children. (Case control study)

Study Number: _____

Subject's Name: _____

Date of Birth / Age: _____

Hospital number :_____

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my child's participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that the Ethics Committee and the regulatory authorities will not need my permission to look at my child's health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my child's identity will not be revealed in any information released to third parties or published. [ ]

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). [ ]

(v) I agree for my child to take part in the above study. [ ]

Signature (or Thumb impression) of the Subject/Legally Acceptable

Date: _____/_____/_____

Signatory's Name: _____ Signature:

Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

Signature or thumb impression of the Witness: _____

Date: _____/_____/_____

Name & Address of the Witness: _____

Department of ENT 2 & Pediatric ENT

CMC Vellore,

Office: 04162282798

CLINICAL RESEARCH PROFORMA

Serial No: _____

Study: To identify and compare microbiological profile of nasopharynx in children with features of symptomatic adenoid enlargement versus asymptomatic normal children- case control study .

Investigator- Dr Joyna Singh

Date of Enrolment: _____

Study 1=Case, 2=Control

Name : Age : Sex : Male(1)/ Female(2)

Hospital number :

Informant: 1=Mother, 2=Father, 3=Self

SES: Modified kuppuswamy score 2019

	Occupation of the head	**Education of the head**	**Monthly family income in Rs**
1	Unemployed -1	Illiterate-1	<3907- 1
2	Elementary occupation-2	Primary school- 2	3908-11,707-2
3	Plant and machine operators and assemblers-3	Middle school- 3	11,708-19,515- 3
4	Craft and related trade workers-4	High school-4	19,519-29,199- 4
5	Skilled agriculture and fishery workers -5	Intermediate or diploma -5	29,200-39,032- 6
6	Skilled workers and shop and market sales workers-6	Graduate - 6	39,033-78,062- 10
7	Clerks -7	Professional or honors- 7	>78,063 - 12
8	Technicians and associate professionals-8		
9	Professionals- 9		
10	Legislators, senior officials and managers- 10		

Upper	26-29
Upper middle	16-25
Lower middle	11-15
Upper lower	6-10
Lower	<5

History

1. Snoring yes/no

2. Mouth breathing yes/no

3. Disturbed sleep yes/no

4. Nasal block yes/no

5. Nasal discharge yes/no

6. Nose bleeding yes/no

7. Voice change yes/no

8. Recurrent URI yes/no

9. Ear discharge yes/no

10. Ear block sensation yes/no

11. Fluctuating hearing loss yes/no

12. History of sore throat/URI in last 4 weeks yes/no

13. Use of antibiotics in the last 4 weeks yes/no

14. Use of nasal steroids yes/no

15. Previous ENT surgeries yes/no

16. No. of people in the house – 2d

17. Siblings with similar complaints yes/no

18. Poor nutrition yes/no

19. History of any allergy yes/no

20. History of immunodeficiency yes/no

Immunisation:

1	BCG	yes/no
2	OPV	yes/no
3	DPT	yes/no
4	MMR	yes/no
5	HiB	yes/no
6	Measles	yes/no
7	Pneumococcal	yes/no
8	Flu	yes/no

Examination

Face

Craniofacial abnormalities: yes/no

Ear

Right : preauricular/ postauricular area normal: yes/no

Discharge : yes/no

Tympanic membrane perforation : yes/no

Left- preauricular/ postauricular area normal: yes/no

Discharge : yes/no

Tympanic membrane perforation : yes/no

Nose

External framework normal : yes/no

Nasal mucosa: Pale mucosa : yes/no

Nasal discharge : yes/no

PNS tenderness : yes/no

Septum: 1=central, 2= Right, 3= Left

Throat

Oropharynx congeation: yes/no

Tonsillar enlargement : yes/no

Oral cavity

Cleft lip : yes/no

Cleft palate : yes/no

X-ray Nasopharynx : 1=GRADE 1, 2=GRADE 2, 3=GRADE 3, 4=GRADE 4

RNE :

A	
C	
E	

Culture growth : 1= No growth

2=Normal flora

3=Streptococcus pneumonia

4=Staphylococcus aureus

5=Haemophilua influenza

6=Group A beta hemolytic streptococci

7=Moraxella cattarahallis

8=Pseudomonas

9=Klebsiella

10=Enterobacter

BIOFILM : YES/NO if yes

1	Non Producers	OD<_ O Db
2	Weak producers	ODb< OD<_ ODb

3	Moderate producer	2ODb<OD<_4Ob
4	Heavy producers	4OD<OD

slno	study	age	sex	inform	occupat	educat	income	ses	snoring	mouthbrt	distslp	nasalblk	nasaldis	nosebld	voicecng	reuri	eardisc	earblk	hearloss	soreuri	antibiot	nslster	entsurg	people	sibling	poornut	allergy	immuno	bcg	opv	dpt
1	1	5	1	1	9	6	1	2	1	1	1	1	1	2	2	1	2	2	1	2	2	1	2	5	2	1	1	2	1	1	1
2	1	4	2	1	8	6	6	2	1	1	1	1	1	2	2	2	1	2	2	2	2	1	2	5	2	2	1	2	1	1	1
3	1	5	2	1	7	6	3	2	1	1	2	1	1	2	1	1	2	1	2	2	2	2	2	3	2	2	1	2	1	1	1
4	1	9	2	1	6	4	4	3	1	2	2	1	2	2	2	1	2	2	2	2	2	1	2	5	2	2	2	2	1	1	1
5	1	8	1	1	7	6	4	2	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	3	2	2	2	2	1	1	1
6	1	6	1	1	6	4	5	2	1	1	1	1	1	2	2	1	2	2	2	2	2	1	2	5	2	2	2	2	1	1	1
7	1	12	2	2	7	5	4	2	1	1	1	1	1	2	2	1	2	2	2	2	1	2	2	5	2	2	2	2	1	1	1
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90	2	8	2	1	5	5	4	3	1	1	2	1	2	2	2	2	2	2	2	2	2	2	2	4	2	2	2	2	1	1	1

slno	study	age	sex	inform	occupat	educat	income	ses	snoring	mouthbrt	distslp	nasalblk	nasaldis	nosebld	voicecng	reuri	eardisc	earblk	hearloss	soreuri	antibiot	nslster	entsurg	people	sibling	poornut	allergy	immuno	bcg	opv	dpt
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