ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

A Prospective Study to Determine the Bacteriology of Palatine Tonsil Surface and Core, along with Histopathological Examination of Tonsil.

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ABSTRACT

Recurrent acute tonsillitis and adenotonsillitis are the main disorders for which the patients visit the general practitioners. The recommended treatment includes prescription of an antibiotic based on superficial tonsillar swab and when the medical treatment fails surgical intervention is undertaken. A thorough knowledge of organism which infects the tonsils is essential for the effective treatment. Hence the present study was undertaken to find the common bacteria in surface and core of the specimen obtained by tonsillectomy and their antibiotic sensitivity patterns along with histopathological examination of tonsil to rule out any associated pathology in department of otorhinolaryngology, IGGGH&PGI, Puducherry. A total of 108 patients were enrolled after getting the informed consent and who satisfy the inclusion and exclusion criteria and this study was carried out from January 2012 to June 2013. A total number of 156 pathogenic isolates was obtained from tonsil surface and core. The most common pathogen isolated was Staphylococcus Aureus followed by Group A β Hemolytic streptococcus and Hemophilus Influenzae. Majority 59.61% was gram positive and gram negative organisms are 40.39 %. Lower antibiotics sensitivity was 63.12% and 36.88% resistant. Whereas 91.38% isolates were sensitive to higher antibiotics and 8.62% isolates were resistant. From the analysis of tonsil surface and core cultures, it is evident that routine culture of the throat by surface throat swab is neither a reliable nor a valid test in the diagnosis of bacterial flora in chronic tonsillitis. Hence, the consideration of a more reliable and valid diagnostic test appears to be necessary. No pathological disease was reported in histopathological examination so doing it as routine for all cases is not feasible unless any suspected evidence to do so.

Key words: Tonsillectomy, Surface and Core Specimen, Antibiotic Sensitivity, Histopathological Examination.

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ISSN: 0975-8585

INTRODUCTION

Tonsillectomy is defined as the removal of the entire Tonsil. It is derived from the Latin word '*tonsilla'*, which means a stake to which boats are tied, and the Greek word '*ektome*', which means excision. Some people have stated that this procedure has been performed for 2000 years [1]. Tonsillectomy and adenoidectomy are among the most commonly performed major surgery in Otorhinolaryngology because of good clinical evidence. Recurrent acute Tonsillitis and Adenotonsillitis are common disorders accounting for substantial percentage of general practitioner visits and financial expenditure annually. The most common operation carried out in children are Tonsillectomy and Adenoidectomy and they continues to constitute a treatment whose benefits in relation to cost and risks have yet to be thoroughly assessed [2]. Despite the ubiquity of the problem, the underlying pathogens are poorly understood. Treatment usually involves prescription of an antibiotic based on a superficial tonsillar swab, with surgical intervention in the event of failed medical treatment [3].

Effective treatment of the child with Tonsillitis depends on the knowledge of the infecting organisms. The practice of swabbing the surface of Tonsil as a culture specimen for determination of the bacteria responsible for the tonsillar pathology may be misleading. Tonsillar disease may stem from the bacteria within the substance of the Tonsil, rather than the bacteria identified on the surface. If the surface culture was representative of the bacteriology of the core, then rational treatment of Tonsillitis could be directed at organisms cultured from surface swabs. Previous studies have shown that surface cultures do not reliably predict core pathogens [4].

Histopathological examination of tonsil was not done in routine as infections and tumors of tonsil are reported rarely. Studies about histopathologial examination of tonsil is limited and never done as routine in our hospital. Hence the present study was conducted to finding the common bacteria in the surface and core of specimens obtained by tonsillectomy and their antibiotic sensitivity patterns, along with histopathological examination of tonsil to rule out any associated pathological disease.

MATERIALS AND METHODS

Study Design: A prospective study

Study site: This study was conducted in Department of Otorhinolaryngology, Indira Gandhi Government General Hospital & Postgraduate Institute, Puducherry.

Study Population and sampling: A total of 108 patients with chronic tonsillitis seeking treatment at Department of Otorhinolaryngology, IGGGH&PGI, Puducherry, who fulfil the inclusion and exclusion criteria were enrolled in this study.

Study variables: Surface Culture, Core Culture, and Histopathological Examination of tonsil.

Study Period: This study was carried out from January 2012 to June2013.

Inclusion Criteria:

1. Recurrent tonsillitis: ≥ 7 episodes in 1 yr or 5 episodes per year for 2 year or 3 episodes per year for 3 years.
2. Chronic tonsillitis (follicular/parenchymal)
3. Quinsy (2nd attack)
4. Age above 5yrs and below 25 yrs

Exclusion Criteria:

1. Patients with any other oral pathology.
2. Revision cases
3. History of any Ear, nose and throat surgery in the past
4. Immuno compromised patients.
5. Tonsil enlargement with stridor / dysphagia
6. Impacted foreign body
7. Systemic indications like Rheumatic fever with arthritis, Sub-acute bacterial endocarditis, Glomerulonephritis , Diphtheria carrier,Chronic bronchitis / bronchial asthma, Phlyctenular conjunctivitis, Urticaria / erythema and failure to thrive.
8. As part of other surgery : Stylalgia (Eagle syndrome), Glossopharyngeal neuralgia, Uvulopalatopharyngoplasty in sleep apnea, Branchial fistula.
9. Chronic medical conditions like diabetes mellitus/hypertension/ischemic heart diseases.

Data Collection:

1. Cases selected for the study will be subjected to detailed history taking and clinical examination.
2. Written informed consent was obtained from all the patients who participated in the study.
3. Ear, Nose and Throat Examination.
4. Diagnostic Nasal endoscopy to note any nasapharynx pathology or anatomical deformity or any sinus pathology.
5. Endolaryngoscopy to know about laryngeal pathology.
6. Investigations: Hemoglobin % , Urine (sugar, albumin, microscopy), Random blood sugar, Blood urea, serum creatinine, HIV,HBsAg , Blood grouping,Culture from the surface of the tonsil /core of the tonsil, Histopathological examination of tonsil.

Method of Collection of Culture Specimens:

All patients underwent tonsillectomy by dissection and snare method. Once the patient is anesthetized, mouth is opened with Boyle Davis mouth gag and fixed with Draffin's bipods. Using a sterile cotton swab, a smear from the surface of right tonsil was obtained. Following this the tonsillectomy procedure was carried out in the same side. The tonsil tissue removed was dipped in povidone iodine solution for 30 to 45 seconds, after which it was rinsed with sterile saline solution, and sectioned into two pieces. The core of the tonsil was then Biopsied,

and this tissue was collected in a sterile glass container and send immediately to the Microbiology laboratory for culture and sensitivity testing.

Direct smear examination:

In Microbiology Department, a thin smear was made on a clean glass slide and was fixed with 95% methanol, by pouring one or two drops on the smear and allowed to act for a minimum of 2 minutes or until the methanol dries on the smear. Gram staining was done for the smears so made and was examined under oil immersion objective to note the various morphological types of bacteria, their number, Gram reaction, presence or absence of inflammatory cells and also to note the numbers of squamous epithelial cells in the sample.

Statistical analysis:

The data was collected further entered and a master chart was created. The collected data were analyzed using Microsoft Office Excel 2007. The data were presented in descriptive statistics and inferential statistics.
Descriptive statistics was explained by frequencies using various data presentation modalities like bar chart. Inferential statistics was carried by using parametric and non-parametric statistical tests wherever applicable depending on the level of measurement of data.

Statistical analysis of results was carried out using Microsoft Office Excel 2007 and the following statistical tests were performed: Chi-square test, kappa (Agreement test), sensitivity and specificity. Level of significance is considered <0.05 for Chi-square test and >0.4 for kappa.

Ethical approval: This study was undertaken after obtaining the ethical approval from institutional ethical committee IGGGH&PGI, Puducherry.

RESULTS

A Prospective study to determine the Bacteriology of Tonsil surface and core was conducted during the period of one and half years, 108 cases were selected randomly and analyzed. Majority of the patients were in the age group of 05-15 years (70.37%) and out of which majority of the cases were females (56.48%) as opposed to males (43.52%). The most common indication for tonsillectomy was recurrent tonsillitis in 73 cases; whereas obstruction caused by enlarged tonsils and adenoids were indications in 23 cases. In 12 cases, recurrent tonsillitis along with obstruction was the indication.

A total number of 178 pathogenic organisms was isolated from 108 cases. More than 1 pathogen was isolated in 49 cases. Staphylococcus aureus was the most common isolate (38%) followed by Hemophilus influenzae (17%) and Group A Beta Haemolytic Streptococcus (GABHS) (16%). The least common pathogen isolated was enterococci (2%). Out of 178 pathogens isolated, 111(62.36%) were gram positive (Staph. Aureus, Staph. Epidermidis, GABHS,

ISSN: 0975-8585

Enterococci) and 67(37.64%) were gram negative (H. Influenzae, Klebsiella, B. Catarrhalis and Pseudomonas). [Table 1]

Table 1: showing pathogenic organisms isolated from the entire cases surface and core organism. (Including non-predominant)

Organism	Number
S. Aureus	67 (38%)
H. influenzae	30 (17%)
GABHS	29(16%)
Klebsiella	17 (10%)
B. Catarrhalis	13(7%)
Pseudomonas	7 (4%)
S. Epidermidis	12 (7%)
Enterococci	3 (2%)
Total	178

Around 156 predominant pathogenic isolates were obtained from the 108 cases studied. Staphylococcus aureus was present only in surface in 12 cases; only in core in 17 cases; and both in surface as well as core in 20 cases. GABHS was present only in surface in 10 cases; only in core in 12 cases; and there were 6 cases isolated both in surface as well as core. H. Influenzae was present only in surface in 6 cases; only in core in 12 cases; and both in surface as well as core in 7 cases. Klebsiella was present only in surface in 10 cases; only in core in 4 cases; and both in surface as well as core in 3 cases. B. Catarrhalis was present only in surface in 6 cases; only in core in 3 cases; and both in surface as well as core in 4 cases. Pseudomonas was present only in surface in 3 cases; only in core in 3 cases; and in 2 cases it was isolated in surface as well as core simultaneously. Staphylococcus Epidermidis was present only in surface in 8 cases; only in core in 4 cases; and both in surface as well as core in 1 case. Enterococci were present only in surface in 3 cases; and it was not isolated from core. The most predominant pathogen isolated was Staphylococcus aureus (32 cases in surface and 37 cases in core). This was followed by GABHS (16 in surface and 18 in core) and Hemophilus influenzae (13 in surfaces and 19 in core). Normal flora was isolated in 28 cases in surface and 31 cases in core. [Table 2]

Table 2: showing distribution of predominant pathogens across surface and core

	Surface	Core	Surface Only	Core Only	Surface+ Core	Total
STAPH.AUREUS	**32**	**37**	**12**	**17**	**20**	**49**
GABHS	**16**	**18**	**10**	**12**	**6**	**28**
H.INFLUENZAE	**13**	**19**	**6**	**12**	**7**	**25**
KLEBSIELLA	**13**	**7**	**10**	**4**	**3**	**17**
STAPH. EPIDERMIDIS	**9**	**5**	**8**	**4**	**1**	**13**
B.CATARRHALIS	**7**	**10**	**6**	**3**	**4**	**13**
PSEUDOMONAS	**5**	**5**	**3**	**3**	**2**	**8**
ENTEROCOCCI	**3**	**0**	**3**	**0**	**0**	**3**
Total						**156**

ISSN: 0975-8585

With regard to comparison of organism obtained from surface and core, in 43 cases, the same pathogen was isolated from surface as well as core. In majority of cases ie in 49 cases, different pathogens were isolated from surface and core. In 6 cases when normal flora was isolated from surface, a pathogen was isolated in core. In 9 cases, when pathogen was isolated from surface, the core organism was found to be normal flora. In 22 cases, normal flora was isolated from both surface as well as core.

The following parameters were calculated using table 3.
Sensitivity = 43.8%, Specificity = 52.4%, Positive predictive value = 45.9%, Negative predictive value = 54.09%.
P=0.79. (Level of significance <0.05) Showing that there is no statistically significant correlation between surface and core cultures.
Kappa (measure of agreement) = 0.037 (significant if >0.4); showing no agreement of results of tonsil surface swab and core tissue cultures.

Table 3: Showing sensitivity and specificity measures

		Core organism		
		Present	**Absent**	**Total**
Surface Organism	**Present**	43 (a)	58 (b)	101 (a+b)
	Absent	55 (c)	64 (d)	119 (c+d)
	Total	98 (a+c)	122 (b+d)	220

Majority of the pathogens were minimally sensitive to common antibiotics (Penicillin, Ampicillin, Cotrimoxazole, Erythromycin and Doxycycline): Staphylococcus aureus (21.5%), Klebsiella (18.5%), Staphylococcus Epidermidis (39.5%) and Hemophilus influenzae (36.5%) and Pseudomonas (20%). Higher sensitivity was shown by Enterococci (93%), GABHS (78%) and Branhamella Catarrhalis (99%). The average sensitivity of all pathogens to common antibiotics was 50.75%. [Figure 1]

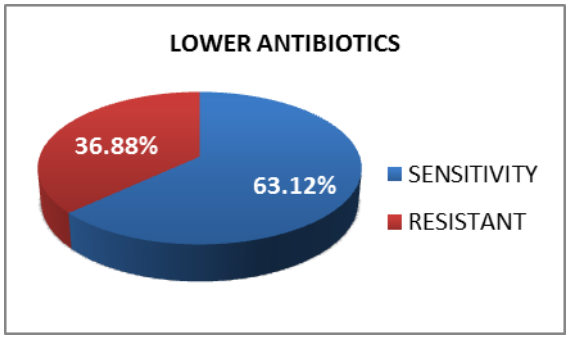

Figure 1: Resistance and sensitivity of lower antibiotics

ISSN: 0975-8585

100% sensitivity was shown to higher antibiotics by Staphylococcus Epidermidis, Hemophilus influenzae, and Branhamella Catarrhalis. This was followed by GABHS (99.5%), Klebsiella (98.5%), Staphylococcus aureus (89%), Enterococci (80%) and pseudomonas (64%). The average sensitivity of all pathogens to higher antibiotics was 91.38% [Figure 2].

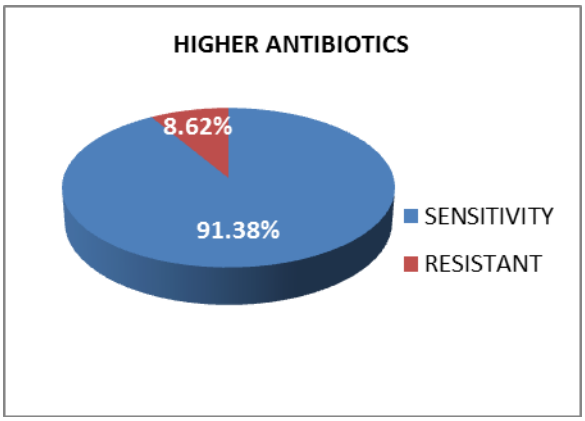

Figure 2: Resistance and sensitivity of higher antibiotics

Histopathological report was sent for examination to rule out any associated infection or tumor, but no cases were reported of any pathological disease. Only 18 cases of chronic non specific tonsillitis, 19 cases of chronic suppurative tonsillitis, 44cases of follicular tonsillitis and 27% lymphoid hyperplasia reported.[Figure 3].

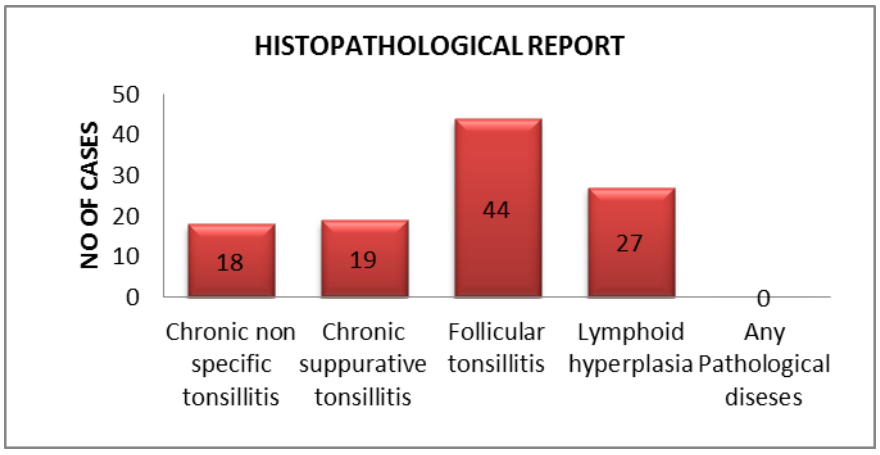

Figure 3: Histopathological report of tonsil specimen

DISCUSSION

Chronic tonsillitis is the commonest disease occurring in younger age group. It is due to chronic inflammation within the tonsils because of insufficient penetration of antibiotics into the core or inappropriate antibiotic therapy. If the surface culture was representative of the bacteriology of the core then rational therapy could be directed at organisms cultured by

surface swab. This study was done primarily to compare between throat swab and tonsil core culture results [5].

In the present study, 70.37% of the patients were in the age group of 5-15 years. Similar observations were made by Gul M et al, in his study in which the age of patients ranged from 3 to 38 years, with a mean age of 12.5 years [6].

With regard to gender, 61% cases were females and 47% were males in the present study. Female preponderance was noted by Kurien M et al study which had 22 females and 18 males [7], where as male preponderance was noted by Gul M et al study, were 54.3% were men and 45.6%were women [6].

The most common indication for tonsillectomy was recurrent tonsillitis,73 cases out of 108 cases, where as obstruction caused by enlarged tonsils and adenoids were indications in 23 cases out of 108 cases in this study. In 12 cases out of 108 obstructions along with recurrent tonsillitis was the indication. In a study by Kumar A et al, recurrent tonsillitis was an indication for tonsillectomy in 42 out of 50 cases. The second most important indication was tonsillar size leading to obstruction in airway or deglutition problem, in 13 cases [8]. Among currently sanctioned indications for tonsillectomy, recurrent throat infection is at once the most frequently invoked [9].

In the present study a total number of 156 pathogenic isolates. More than 1 pathogen was isolated in 49 cases. Staphylococcus aureus was the most common isolate (31.41%) followed by GABHS (17.95%) and Hemophilus influenzae (16.03%) and the least common pathogen isolated was enterococci (1.92%). Out of 156 pathogens isolated, 93(59.61%) were gram positive (Staph. Aureus, Staph. Epidermidis, GABHS, Enterococci) and 63(40.39%) were gram negative (H. Influenzae, Klebsiella, B. Catarrhalis and Pseudomonas). Similar observations were made in previous studies as expressed in table 6.

Table 6: showing comparison of common organisms isolated with previous studies

	Present study	**Gul M et al [6] 2007**	**Abdulrahman AS et al. [5] 2004**
First common pathogen	Staphylococcus aureus (31.41%)	Staphylococcus aureus (33.6%)	Staphylococcus aureus (77.7%)
Second common pathogen	GABHS (17.95%)	GABHS (31.1%)	GABHS (18.5%)
Third common pathogen	Hemophilus influenzae (16.03%)	Haemophilus influenzae (14.2%)	E. Coli (3.7%)

In the present study, in 43 cases, the same pathogen was isolated from surface as well as core. In majority of cases (49), different pathogens were isolated from surface and core. In 6 cases when normal flora was isolated from surface, a pathogen was isolated in core. In 9 cases,

when pathogen was isolated from surface, the core organism was found to be normal flora. In 22 cases, normal flora was isolated from both surface as well as core. Statistical analysis was done to determine the usefulness of surface swab in predicting the core organism by which; The Sensitivity was found to be 43.89%, Specificity: 52.45%, Positive predictive value: 48%, Negative predictive value: 49%, with an overall accuracy of 48.5%. There is no statistically significant correlation between surface and core cultures (P value>0.05). Kappa (measure of agreement) was <0.4, showing no agreement of results of tonsil surface swab and core tissue cultures. Similar observations were made in previous studies as expressed in table 7.

Table 7: showing comparison with previous studies regarding surface-core relation

Tonsillar surface	Tonsillar core tissue	Present study	Gul M et al [6] 2007	Kurien M et al [7] 2000	Surrow et al [4] 1989
Normal flora	Normal flora/ No growth	22%	33.62%	15%	14.4%
Normal flora	pathogen	6%	12%	25%	43.3%
pathogen	Same pathogen	43%	21.5%	30%	33%
Pathogen	Different pathogen	49%	31%	15%	5.1%
pathogen	Normal flora	9%	1.7%	15%	4.1%

Majority of the pathogens were resistant to common antibiotics (Penicillin, Ampicillin, Cotrimoxazole, Erythromycin and Doxycycline). Staphylococcus Epidermidis (39.5%), Hemophilus influenzae (36.5%) Staphylococcus aureus (21.5%), Pseudomonas (20%) and Klebsiella (18.5%), showed the least sensitivity. Whereas higher sensitivity was shown by Branhamella Catarrhalis (99%), Enterococci (93%) and GABHS (78%) to common antibiotics. 63.12% isolates were sensitive to lower antibiotics and 36.88% isolates were resistant to lower antibiotics.

All pathogens except pseudomonas (64%), showed high sensitivity to higher antibiotics (Gentamycin, Ciprofloxacin, Ofloxacin, Cefotaxime and Amikacin). 100% sensitivity was shown by Staphylococcus Epidermidis, Hemophilus influenzae, and Branhamella Catarrhalis. This was followed by GABHS (99.5%),Klebsiella (98.45%), Staphylococcus aureus (89%), Enterococci (80%). 91.38% isolates were sensitive to higher antibiotics and 8.62% isolates were resistant to higher antibiotics.

In a study on “Present situation of antibiotic resistances in tonsillar infections” by Maroto PD et al [10], The highest resistances were for the S. aureus (penicillin 91%, erythromycin 18% and 5% to the rest of the beta-lactams), followed by H. influenzae (50% clarithromycin, 30% amoxycillin and 2% cephalosporins) and S. pyogenes (28% erythromycin, 10% clindamycin and 3% penicillin). The authors noticed minimal resistance to cephalosporins, and for this reason they appear to be the safest option, except in children under five years old, in which Amoxycillin is still the first line treatment, because the causative agent is S. pyogenes, sensitive to that antibiotic.

In the study by Abdulrahman AS et al [5], most staphylococcus aureus strains were resistant to Ampicillin (85.71%), followed by Amoxycillin Clavulanic acid (80.95%). Regarding GABHS strains, only one strain was sensitive to Amoxycillin Clavulanic acid (20%) while all are resistant to Ampicillin (which differs from the present study), followed by Erythromycin, Amoxycillin and Cefpodoxime. The gram negative isolates were sensitive to Cefpodoxime and Trimethoprim Sulphamethoxazole and resistant to Amoxycillin Clavulanic acid, Amoxycillin, Dalacin and Ampicillin.

Antibiotic resistance is a global public health problem. It is more frequently encountered in hospital acquired pathogens; however the incidence of antibiotic resistant pathogens in the community acquired infections has also been in the rise in recent years. Over use of antibiotics and consequent antibiotic selective pressure is thought to be the most important factor contributing to the appearance of different kinds of resistant bacteria [11].

Histopathological examination of tonsil was sent for all cases of tonsillectomy done at our hospital. No pathological disease (0%cases) was reported, so unless there is any clinical evidence of asymmetrical enlargement of tonsil or any suspected disease sending sample for histopathological examination will reduce unnecessary added cost and loss of man power.

CONCLUSION

Tonsillectomy remains one of the most common surgeries performed in children. The majority of the patients were between 5 to 15 years. Recurrent tonsillitis was the most common indication for surgery. Out of the total One hundred and eight cases studied, a total no. of 156 pathogenic isolates was obtained from tonsil surface and core. More than 1 pathogen was isolated in 49 cases. Normal flora was obtained in 37 cases. The most common pathogen isolated was Staphylococcus Aureus followed by Group A β Hemolytic streptococcus and Hemophilus Influenzae. Majority (59.61%) was gram positive and gram negative organisms are 40.39 %.

In account for sensitivity testing, 63.12% isolates were sensitive to lower antibiotics and 36.88% isolates were resistant to lower antibiotics. Whereas 91.38% isolates were sensitive to higher antibiotics and 8.62% isolates were resistant to higher antibiotics, showing that a complete course of a higher antibiotic may be tried before planning for tonsillectomy. From the analysis of tonsil surface and core cultures, it is evident that routine culture of the throat by surface throat swab is neither a reliable nor a valid test in the diagnosis of bacterial flora in chronic tonsillitis. Hence, the consideration of a more reliable and valid diagnostic test appears to be necessary.

No pathological disease was reported in histopathological examination so doing it as routine for all cases is not feasible unless any suspected evidence to do so.

ISSN: 0975-8585

ACKNOWLEDGEMENT

I would like to thank Dr.R.Sachidanandam (Retd. HOD, Dept of Otorhinolaryngology, IGGGH&PGI), Dr.K.Sarangapani (HOD, Dept of Microbiology, IGGGH&PGI), Dr.M.Karunanidhi (Dept of Otorhinolaryngology, IGGGH&PGI) for their support and valuable suggestions during the study.

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