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ORIGINAL ARTICLE

Fungal Infection as Cause of Chronic Tonsillitis; A Histopathological-microbiological Study

[1]Maha K. Gohar*, [2]Samir S. Sorour, [2]Wail Fzyez, [2]Mohammed K. Mobasher, [3]Hesham R. Abd El Aziz
[1]Medical Microbiology & Immunology, [2]Otorhinolaryngology, [3]Pathology Departments
Faculty of Medicine Zagazig University

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Corresponding Author:
Maha K. Gohar
Assistant Professor of Microbiology and Immunology
Faculty of Medicine, Zagazig University, Egypt
goharmaha@gmail.com
+2 0100 010 1819

ABSTRACT

Oral fungal infections are common opportunistic infections that may take different forms. Fungal infection as a cause of chronic tonsillitis indicates tonsillectomy as treatment, so preoperative identification of fungal causes of tonsillitis is valuable. ***Objectives:*** *The aim of this study was to assess the incidence of fungal infection in chronic tonsillitis using preoperative microbiological basis and post-tonsillectomy histopathological basis.* ***Methodology:*** *This study included 75 children (patients group) suffering from chronic tonsillitis, and 15 healthy children complaining from obstructive sleep apnea (OSA) and snoring with no history of chronic tonsillitis. Each child subjected to full history taking, clinical examination and preoperative investigations. Tonsillar swabs were taken from each tonsil of all subjects. Smears were stained and examined microscopically then cultured using Sabouraud dextrose agar (SDA). Post-tonsillectomy, histopathological examination was carried out.* ***Results****: Tonsillomycosis is significantly associated with dental caries and recurren acute attacks of tonsillitis (with p=0.004 & 0.001respectively). Hypertrophic tonsil and neutropenia are significantly detected in tonsillomycosis (p=0.001 for both). A histopathological examination found that fungal invasion of tonsillar tissue was found in about 31.3% of tonsils (47/150) with immune reaction and presence of chronic inflammatory cells. Preoperative microbiological work revealed tonsillomycosis in 45/150 (30%) of tonsils. In comparison to histopathology, microbiological examination had 95.9% sensitivity, 100% specificity, 100% PPV and 98.1% NPV.* ***Conclusion****: Fungal infection is a common cause of chronic tonsillitis. Haphazard use of antibiotics and dental caries has a significant predisposing role in tonsillomycosis. Neutropenia and hypertrophic tonsils can be taken as an indicator for tonsilar fungal infection. Microbiological examination is valid for preoperative diagnosis of tonsillomycosis.*

INTRODUCTION

Fungi are common opportunistic infection of the oral cavity, oropharynx, and corners of the mouth. Fungi are known to be in a commensal state and can been shifted to pathogenic state[1]. Risk factors for development of pathogenic form include local and systemic factors. Many factors which altered ecology of the region such as topically delivered drugs (corticosteroids), xerostomia (with loss or diminution of saliva's protective function), passive smoking, and dental problems are known to favor establishment of infection. Children that have some serious immune or metabolic defect, or have systemic condition such as poorly controlled diabetes mellitus or immunosuppressive condition are also at risk [2,3].

In a healthy child, infection is usually self-limited, however latency is commonly established, rather than eradication of infection. Reactivation of latent infection may then occur if the individual becomes immunosuppressed[4].

Local Common presentation of tonsillomycosis include: redness and/or swelling, white or yellow patches on the tonsils, tender, stiff, and/or swollen neck, sore throat and painful or difficult swallowing. Tonsillomycosis possibly causes tonsillar hypertrophy causing variable degrees of upper airway obstruction [5].

Candidiasis is the most common fungal infection of the oral cavity; there are also six noncandidal oral infections: aspergillosis, cryptococcosis, histoplasmosis, blastomycosis, paracoccidioidomycosis, and zygomycosis (mucormycosis) [6].

The aim of this study was to assess the predisposing factors and incidence of fungal infection in chronic tonsillitis using preoperative microbiological examination and post-tonsillectomy histopathological work.

METHDOLOGY

Subjects:

- Patients group: this include 75 children (32 males and 43 females) complaining from chronic tonsillitis. Full history were taken from this group including: age, sex, history of indication for surgery, presenting

symptoms, predisposing factors (drug intake as corticosteroids or antibiotics, dental caries, immunosuppressive conditions and diabetes mellitus) and number of attacks.

- Control group: 15 healthy children (9 males and 6 females) complaining from obstructive sleep apnea (OSA) and snoring with no history of chronic tonsillitis (control group).
  - This study was carried out in Otorhinolaryngology and Medical Microbiology and Immunology Departments, Zagazig University, Egypt between February 2013 and December 2015. Verbal consents were obtained from parents or guardians. This study was approved by the IRB Zagazig University.

Sampling:

1. Tonsillar swabs were obtained from each participant using sterile swabs and sterile tongue depressor for squeezing of tensile crypts to obtain materials deep in it, and for scrapping of the patches. Samples were sent to Microbiology and Immunology Department.
2. Blood samples for preoperative work up.
3. After tonsillectomy, tonsils were sent for histopathological examination.

Methods:

Microbiological examination:

- Two swabs were rolled on two slides. The first slide was stained by Lactophenol cotton blue stain and the other by Calcofluor white stain. The later was examined under ultraviolet microscope.
  - Candida species showed rounded and oval budding yeast cells with hyphea and pseudohyphea.
  - Aspergillus species showed septate hyphea of uniform width and V- shaped branching.
  - Rhinosporidium showed sporangia.
- Other two swabs were rolled on the surface of two Sabouraud dextrose agar plates Sabouraud Dextrose Agar (SDA –Difco Laboratories, Detroit, MI, USA) with chloramphenicol (Sigma, St Louis, MO, USA). One plate was incubated at 37ºC and the other at room temperature.
- *Identification:* Yeast colonies were identified by gram stain and germ tube test, while molds were identified by both macroscopic and microscopic examination using cellophane tape method and slide culture technique, using potato dextrose agar medium[7] (Fig 1&2). Vitek MS MALDI- TOF fungal identification system was used for identification of isolates to species level, using disposable target slide (product no. 111149 BM; bioMerieux, Marcy l' Etoile, France) and according to manufacturer instructions.

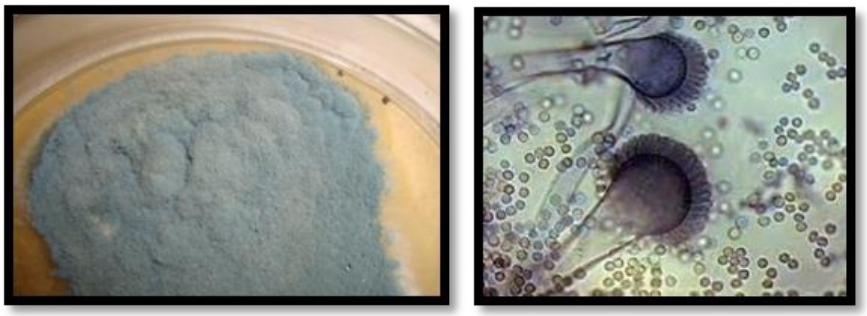

Fig. 1: *Aspergillus fumigatus* on SDA and under microscope by cellophane tape method (x 400).

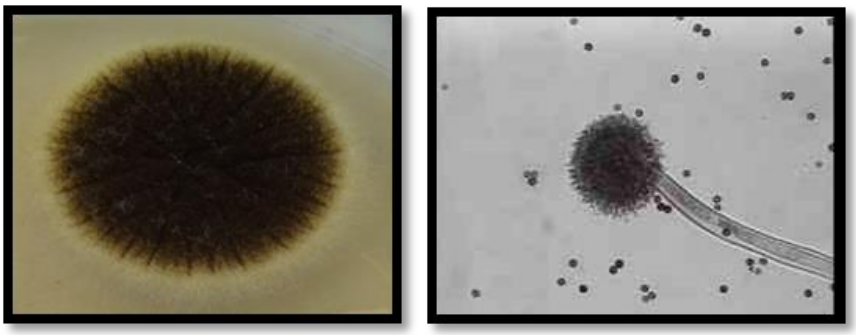

Fig. 2: *Aspergillus niger* on SDA and under microscope by cellophane tape method (x400).

Histopathological examination:

Tonsillectomy was performed with dissection or bipolar diathermy. Soon post-operatively, the size of two tonsils from each subject was measured (length×width). The bisected tonsils were immediately preserved in 10% neutral buffered formalin in separate bottles and then sent to Histopathology Department as they were dehydrated, embedded and cut at 4-5 micron thick by routine methods. The sections were stained with Hematoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS). The assessment of each tissue sample included evaluation of mucosal surface, active tonsillitis, cryptitis and location of the *fungus* elements**.** The (H&E) sections were examined under ultraviolet microscope (340 nm wave length) for autofluorescence of fungi.

Statistical analysis:

The obtained results were statistically analyzed by using SPSS version 17. Data were expressed as Mean ± SD for qualitative variables, number and percentage for categorical variables. Chi-Square (X^2) and student t-test at appropriate. P<0.05 was considered statistically significant.

RESULTS

Our study included 75 patients there age range was 4- 12 years and mean (6.3±2.2). They were 43 males (57.3%) and 32 (42.3%). We found tonsillomycosis in 24/75 (32%) patients and 51/75 (68%) of patient had no fungal element or tonsillomycosis. Histopathological examination detected tonsillomycosis in 47/150 (31.3%) tonsil specimens, however microbiological examination revealed it in 45/150(30%).

Microbiological results:

We revealed fungal element in 45/150 specimens by microscopic examination and in 39/150 specimens by fungal culture. Six cases of Rhinosporidium were detected by microscopic examination and missed by culture. Thus both techniques revealed tonsillomycosis in 45/150 specimens of tonsils (table 1).

Table 1: show results of microscopic examination vs fungal culture:

	Microscopic results	***Cultural results***
Candida species	**21**	**21**
Molds	**18**	**18**
Rhinosporidium	**6**	**0**
Total	**45**	**39**

As regard species identification of the isolates, *Candida albicans* was the most frequent cause of tonsillomycosis that represent 18 (40%), followed by *Aspergillus fumigates* 12 (26.7%) and lastly *Candida krusi* 1(2.2 %) as in table (2)

Table 2: Shows the results of identification tests:

Fungal species	**No (%)**
Candida species (21)	
C. albicans	18 (40 %)
C. glabarta	2 (4.4%)
C. krusi.	1 (2.2%)
Molds (18)	
Aspergillus fumigates	12 (26.7%)
Aspergillus niger	6 (13.3 %)
Rhinosporidium (6)	
Rhinosporidium seeberi	6 (13.3 %)
Total	45(100 %)

All tonsils of control group were negative for significant fungal finding either microscopically or by culture.

Histopathological results:

Histopathological examination after tonsillectomy revealed that 47/150 (31.3%) specimens were positive for fungal infection.

Macroscopic findings:

Patients groups showed tonsils with variable sized crypts filled with cell debris and necrotic material on the surface of the tonsils. The control group showed large size crypts without cell debris and necrotic material on the surface of the tonsils.

1. 135 tonsils (105 tonsils from the patient group and 30 tonsils from the control group) were hypertrophied with a size ranging from 3.5 to 7cm in diameter.
2. 45 tonsils from the patient group were atrophied with a size ranging from 0.5 to 2.0 cm in diameter.

Microscopic findings:

- All participants revealed reactive follicular hyperplasia (Fig 3).

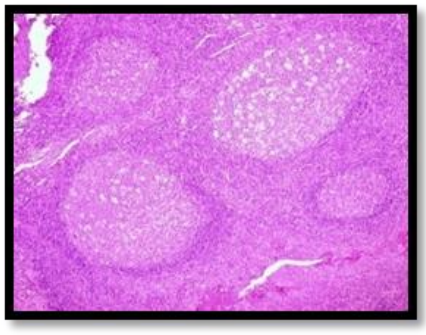

Fig. 3: Photomicrograph of tonsil showing reactive follicular hyperplasia with numerous variable-sized germinal centres with large numbers of tangible body macrophages surrounded by a collar of small resting naive B cells. H&E x 100

- In patient group:

1. 21/150 tonsils (14%) revealed the picture of Candidiasis with prominent blastopores (Fig 4).

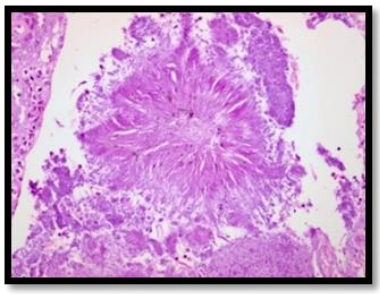

Fig. 4: Photomicrograph of tonsil showing blastopores of Candidal fungal colonies PAS x 400.

2. 18/150 tonsils (12%) revealed the picture of Aspergillosis with septate hyphae (Fig 5).

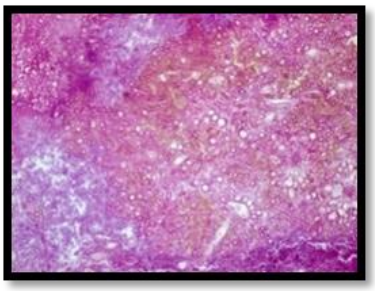

Fig. 5: Photomicrograph of tonsils showing septate hyphae of Aspergillus fungal colonies, PAS x 400.

3. 8/ 150 tonsils (5.3%) revealed the picture of Rhinosporodiosis with Sporangia 100-200 um in diameter (spherules) of Rhinosporidium seebreri containing sporangio spores in different stages of growth (Fig 6).

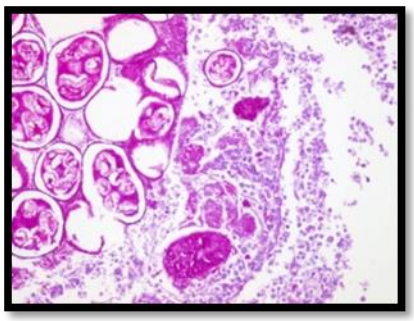

Fig. 6: Photomicrograph of tonsil showing numerous variable-sized cysts of Rhinosporiodosis fungal colonies, H&E x 400.

There was very little cellular response to the organisms in the form of few numbers of mononuclear cells and scanty proteinaceous debris.

4. The adjacent epithelium was normal except for minimal hyperplasia and small number of neutrophils.

The obtained results were demonstrated in the following tables and were divided into three types of data:

I: Factors predisposing for fungal infection

The factors were represented statistically in table **(3)**. Our results revealed that, fungal infection of tonsils is significantly associated with dental caries and patients with history of more than 5 attacks of tonsillitis in one year (*p* values are 0.004 and 0.001 respectively).

Table 3: Factors associated with chronic fungal infections of tonsils.

Factors	Without fungal infection(51)		With fungal infection (24)		X^2 or t	*P*
Age (years)						
X ± SD	6.2 ± 1.0		6.3 ± 2.3			0.79
Range	(5-8)		(4-12)		0.26	(NS)
Sex (n)	No	%	No	%		
Male (32)	22	68.75	10	31.25	0.03	0.9
Female (43)	29	67.4	14	32.6		(NS)
Dental cares (n)♦						
Absent (35)	29	82.9	6	17.1		0.004*
Present (40)	22	55.0	18	45.0		(S)
No. of attacks of AT (n)♦						
≤5 in one year (39)	35	89.8	4	10.2	0.8	0.001*
>5 in one year (36)	16	44.5	20	55.5		(S)

AT=Acute tonsillitis n= Number of cases NS=Non Significant S= Significant
♦=According patient history.

II: Neutropenia and tonsils size:

The relation between tonsil size and fungal infections were shown in table (**4**).

Table 4: Relation between size of tonsils and tonsilloymcosis

	Without tonsilloymcosis (N= 103)	**With tonsilloymcosis (N=47)**	***p***
Size of tonsil	2.96 ± 1.1cm	4.8 ± 1.7cm	0.001(S)

With Chi-square equation and student-t test, probability value revealed that hypertrophy of the tonsil is highly significant (p =0.001) in tonsillomycosis.

Table (5) shows the association between neutropenia and tonsillomycosis.

Table 5: Relation between tonsilloymcosis and neutropenia

	Without tonsilloymcosis (51)	***With tonsilloymcosis (24)***		
	No (%)	**No (%)**	**X^2**	***P***
No neutropenia (40)	40 (78.43)	0.00 (0.00)	3.92	0.001
With neutropenia(35)	11 (21.57)	24 (100.0)		(S)

From investigation profile, pre-operative CBC of our 75 patients revealed 35 (46.7%) of them had neutropenia. All tonsilloymcosis patient were neutropenic (24/24) 100%. And thus as regard association between fungal infection and neutropenia, it was highly significant (p = 0.001).

III: Validity of microbiological work before tonsillectomy.

A comparison between microbiology and histopathology in detection of fungal infection was shown in table (6).

Table 6: Validity of microbiology methods in detection of fungal infection in comparison with histopathology in patients:

	Histopathology		
	+ve	**-ve**	**Total**
Microbiology			
+ve	45	0	45
-ve	2	103	105
Total	47	103	150

Sensitivity= 95.9% Specificity= 100% PPV= 100% NPV= 98.1%.

DISCUSSION

Althaugh candidiasis is the most common fungal infection of the oral cavity; there are six noncandidal oral infections: aspergillosis, cryptococcosis, histoplasmosis, blastomycosis, paracoccidioidomycosis, and zygomycosis (mucormycosis). Although these noncandidal fungal infections are considerably less common than oral candidiasis, they commonly produce subclinical infection[6].

In the present study, histopathologically identification of fungal colonies in tonsillar tissue with general and specific fungal stains (Hematoxylin & Eosin and Periodic Acid Schiff Stain) come in agreement with the histopathological results of Maher et al.[8] but with higher incidence. This study revealed 47/150 (31.3%) of tonsils had tonsillomycosis, 21/150 tonsils (14%) showed morphological character of candidiasis and 18/150 (12%) of tonsils showed morphological character of Aspergillosis and 8/150 (5.3%) of tonsils had Rhinospordiosis.

Rhinosporidium is non culturable fungus and could be detected only in direct microscopic examination of grey patches due to bulging of the sporangia through the attenuated epithelium or during histopathological examination[9]. In our study, histopathological examination revealed 8 tonsils with Rhinosporidium within tonsillar tissue versus 6 tonsils by microbiological work. Microscopic examination of microbiology work coulden't detect any Rhinosporidium elements in the scrapped patches of these two tonsils, this may be due to non pulging of sporanigia and limitation of infection to deep tonsillar tissue that could be detected in histopathology sections only. Consequently, microbiological work before tonsillectomy (microscopic and culture) revealed 45 tonsils with fungal infection, however histopathological examination after tonsillectomy revealed 47 tonsils, giving microbiological work 95.9%, 100%, 100% and 98.1% sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) respictively.

This study considers actinomycosis, gram positive saprophytic bacteria and tonsillar crypts are its normal habitat as suggested in Grüner study[10].

This study found a significant relation between neutropenia and fungal infection of tonsil in agreement with, Scully and his colleagues[11]. They explained their finding as polysaccharide antigen of some fungal

species as *C. albicans* may generate complex sequence of interaction that suppress T , B and other immune cells which slow inflammatory process in immuncompetent person. In another study by McIntyre, he attributed the neutropenia to the systemic effect of fungal infection on bone marrow [12& 13].

In our study dental caries, as indicator of bad oral hygiene, was significantly associated to tonsillomycosis. This is in accordance with Farah et al., study who stated that candida count are reduced in oral cavity by tooth brushing moreover denture as prostheses promote the presence and growth of fungi in oral cavity.

This study found significant relation between number of attacks with acute tonsillitis per year and fungal infection. This can be attributed to the blind and recurrent uses of broad spectrum agents which are active against wide range of bacteria. Antibiotic suppress the normal bacterial flora, which control fungal levels in oral cavity of healthy individuals, through competition for dietary substrates and epithelial cell adhesion. Moreover, some antibiotics have been shown to impair the immune response by affecting neutrophil activity [14& 15].

In this study tonsillomycosis was significant in children with: hypertrophic tonsils with the mean size 4.8±1.7 cm in diameter. Tonsillar enlargement can be attributed to that, some fungal component acts as antigens that induce epithelial hyperplasia and infiltrated with few neutrophils without any granulomatous reaction . In contrast, the study of Jokinen[16] and his colleagues found chronic tonsillomycosis is significantly associated with atrophied tonsils.

CONCLUSION

This study concludes that, long term antibiotic administration due to repeated attacks of acute tonsillitis and dental cares are predisposing factor for tonsillomycosis. *Candida albicans* is most frequent fungal cause of tonsillomycosis. Neutropenia is a definite finding in patients with tonsillomycosis. Consequently this study recommends microbiological examination of recurrent tonsillitis before tonsillectomy decision. Also, otorhinolaryngological specialist should be trained to prompt recognition of fungal tonsiller infections.

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