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Original Article

Pathology Section

Histopathological Reporting of *Actinomyces* Colonies in Tonsils-Utility or Futility? A Retrospective Study

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ABSTRACT

Introduction: *Actinomyces* colonies in tonsillectomy specimens are often ignored as commensals and histopathology reports do not carry any information about them. But evidence suggests that under favourable conditions these may become invasive and pathogenic, serving as precursors to later systemic disease. Early identification of mucosal breach by these bacteria may have a role in the clinical management and future prognosis.

Aim: To determine the frequency of occurrence of *Actinomyces* colonies in tonsillectomy specimens and study the various associated histological alterations which may provide a clue to their pathogenicity.

Materials and Methods: This was a retrospective study on 140 tonsillectomy specimens, from 70 patients who had undergone elective, bilateral tonsillar surgery. Tonsils were examined for *Actinomyces* colonies and histological alterations as per two different grading criteria. Different clinicopathological features were associated, those with and without *Actinomyces* colonies using appropriate statistical tools (t-test, Chi-square and Fisher's exact test; Statistical Package for the Social Sciences {SPSS} version 23.0).

Results: A total of 140 tonsillar tissue specimens were analysed from 70 patients and divided into two groups Group 1 (with actinomycosis) and Group 2 (without actinomycosis) with a mean age of 13.6±6.63 years and 11.8±7.19 years, respectively. The frequency of *Actinomyces* colonies was found to be 40% (28 of 70 patients) with bilateral presence in 28.5% (8 of 28 patients). Tissue reaction was present in 36.11% (13/36 tonsillar specimens) of the tonsillectomy specimens harbouring *Actinomyces*. Deeply situated colonies elicited tissue reaction more frequently (11/19 tonsillar specimens; 57.89%) compared to superficial ones (2/17 tonsillar specimens; 11.76%). Tonsillar cryptitis was the most statistically significant histopathological finding associated with the presence of *Actinomyces* colonies (p=0.029).

Conclusion: Tonsillar cryptitis was found to be a consistent histological finding in tonsillar actinomycosis. The frequent acute inflammatory responses generated by deeply located *Actinomyces* colonies refutes their role as mere commensals. It is suggested that tonsil histopathology reports should mention the presence/ location/ tissue reaction surrounding *Actinomyces* colonies to differentiate mere colonisation from invasion. This could have profound clinical implications in terms of necessity of antibiotic use for prophylaxis/ and or treatment.

Keywords: Commensals, Histopathology, Palatine tonsils

INTRODUCTION

Tonsillectomy is a common surgical procedure in children and young adults with chronic tonsillitis being the most common cause [1]. *Actinomyces* colonies are often encountered in tonsillectomy specimens but their exact role in the aetiopathogenesis of tonsillar diseases is still unclear. While some have come out in support of an etiological role for this organism in adenotonsillar diseases others have implicated its role as a mere saprophyte and commensal of the normal tonsil [2-6]. There are about 30 species of *Actinomyces*, of which about eight can be found in humans [7]. *Actinomyces (A)* that cause human disease are not found in nature but are constituents of the commensal bacterial flora of the oropharynx, gastrointestinal tract, female genital tract. Hence, this is not an exogenous infection and no person to person spread occurs. The *A. israeli* and *A. naeslundii* are the most common type which can cause human infection. Thus, it's clear that under appropriate, favourable circumstances these commensals have the capability to turn pathogenic [8,9]. But the presence of these bacterial colonies in tonsillar tissue sections is ignored by most pathologists as normal bacterial commensals and it finds no mention in tonsil histopathology reports.

For definitive treatment of tonsillar actinomycosis, a prolonged course of antibiotics is recommended even after tonsillectomy has been done [10,11]. The clinical implication of detecting its presence, identifying local tissue reaction in tonsillar specimens lies in the fact that in the absence of an adequate postoperative antibiotic therapy, there is a likelihood of its recurrence and relapse in certain susceptible individuals as some other form like oral or cervicofacial actinomycosis. There have been some rare but well documented autopsy cases of fatal complications following aspiration of these bacterial colonies from the oropharynx into lungs leading to cardio-respiratory complications and sudden death [12]. Identification of these early penetrated sites of tonsillar infection beds and treatment with appropriate antibiotic prophylaxis even post-tonsillectomy can lower the possibilities of later widespread complications [13]. The present study was undertaken to find the frequency of occurrence of *Actinomyces* colonies in tonsillectomy specimens and evaluate histologically to ascertain if these colonies are associated with any specific histological alterations in the tonsillar milieu which may be a cue to their possible pathogenicity.

MATERIALS AND METHODS

This was a retrospective study carried out in an eight month period (January 2019 to August 2019) and data analysed from February 2021 to March 2021 at the Department of Pathology in a tertiary healthcare centre in Ajmer, Rajasthan (India) after Institutional Ethical Committee approval (296/Acad-III/MCA/2021 (15/2/2021).

Inclusion criteria: Tonsillectomy specimens from all the elective tonsillar surgeries performed for either tonsillar hypertrophy or recurrent tonsillitis.

Exclusion criteria: Tonsillar biopsies and tonsillectomies for malignant lesions were excluded.

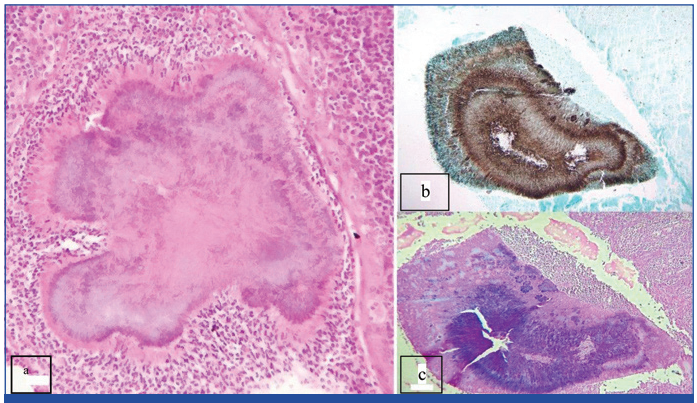
All cases that met the inclusion criteria during the study duration were included and analysed as part of the study. A total of seventy patients

underwent bilateral elective tonsillectomy during this period (140 tonsillar specimens). Relevant clinical data like age, gender of the patient, clinical indications for tonsillectomy were recorded from the histopathology requisition forms.

Duly labelled jars containing right and left tonsils were subjected to gross examination that included noting external features and measurements in three dimensions. Representative pieces, two from each tonsil were subjected to routine tissue processing. Tissue sections ((4-6 micron thickness) were stained by Haematoxylin and Eosin (H&E) stain and studied under light microscopy (Labomed Vision 2000).

The usual pattern of tonsillectomy reporting in the institute is either 'chronic non-specific tonsillitis' or 'lymphoid hyperplasia of tonsillar tissue'. For the purpose of study, a parallel, detailed examination was done to note histological alterations in tonsillar architecture that included mucosal surface, crypts and associated lymphoid tissue. The tonsil sections were thoroughly examined for presence of *Actinomyces* colonies. *Actinomyces* colonies were easily identified microscopically. [Table/Fig-1] by their characteristic appearance as aggregates of filamentous microorganisms arranged in a radial spoke-like fashion, the so called 'ray-fungus' [2,4,5]. They were studied for their presence, location (lying superficially or deep within crypts) and tissue reaction in the form of outer zone of granulation tissue, central necrosis surrounded by an acute inflammatory infiltrate [Table/Fig-1]. The histopathological features of tonsillar specimens with and without *Actinomyces* were evaluated and compared by applying two different histologic grading modalities by Reis LG et al., and Ugras S and Kutluhan A [Table/Fig-2,3] [14,15]. The tissue sections were studied by two pathologists and the final consensus reached was recorded.

A clinicopathological correlation was done between cases with and without *Actinomyces*. To determine age related relationship with the presence of *Actinomyces*, a cut-off of 20 years and less to include children and adolescents and more than 20 years to include adults was taken. For evaluation of the histological criteria by Reis LG et al., the various histological variables were studied for their presence and subjectively graded in to different microscopic levels of severity signifying absent (level 0), mild (level 1), moderate (level 2), severe (level 3) in appropriate magnification fields as shown in [Table/Fig-2] [14]. The sections were simultaneously studied for the eight histological criteria stated by Ugras S and Kutluhan A as shown in [Table/Fig-3] [15]. The results were tabulated separately to analyse for any statistical association.



[Table/Fig-1a-c]: *Actinomyces* colonies with the characteristic 'sun-ray appearance' and Tissue-reaction surrounding the colonies.
1a-H&E stain (x100); 1b-Gomori's Methamine Silver stain (x100); 1c- Gram's stain (x100)

Histological feature	Grading	
Lymphoid follicles (studied and graded under 40x magnification)	0	Predominance of primary follicles
	1	Predominance of secondary follicles
	2	Primary and secondary follicles in similar proportions
Number of Lymphoid follicles (Average of 5 fields in 40x magnification)	1	< 25 follicles per field
	2	>25 follicles per field
Number of Germinal centres (Average of 5 fields in 100x magnification)	1	< 6 germinal centres per field
	2	>6 germinal centres per field
Necrosis and Fibrosis subjectively classified in the most intense focus (in 100x magnification)	0	Absent
	1	Mild
	2	Moderate
	3	Severe
Crypt epithelium was studied and subjectively graded for neutrophilic permeation, reticulation and plasma cell infiltration (in 400x Magnification)	0	Absent
	1	Mild
	2	Moderate
	3	Severe

[Table/Fig-2]: Histologic grading modality by Reis LG et al., [14].

Histopathological criteria
Presence of slight-moderate lymphocyte infiltration (SMLI) in the surface epithelium
Presence of small or large intraepithelial tight aggregates of lymphocytes leading to the defect in the surface epithelium (Ugras's abscess)
Presence of diffuse lymphocyte infiltration (DLI) leading to the defect in the surface epithelium
Presence of polymorphonuclear leukocytes (PL) in the surface epithelium and in the sub epithelial area
Presence of lymphoid hyperplasia (LH)
Increase in the plasma cells number (IPCN) in the sub epithelial area and in the interfollicular area
Presence of fibrosis (F)
Presence of atrophy (A).

[Table/Fig-3]: Histopathologic criteria given by Ugras S and Kutluhan A [15].

STATISTICAL ANALYSIS

The data was statistically analysed for association with age, gender and different histological variables between the two groups. Relationship with quantitative variable like age is expressed as mean±SD and t-test was used. For qualitative (categorical) variables like gender, various histological criteria, Chi-square and Fisher's exact test was used as appropriate. Probability levels of less than 0.05 were considered significant. The SPSS software program version 23.0 was used for statistical analysis.

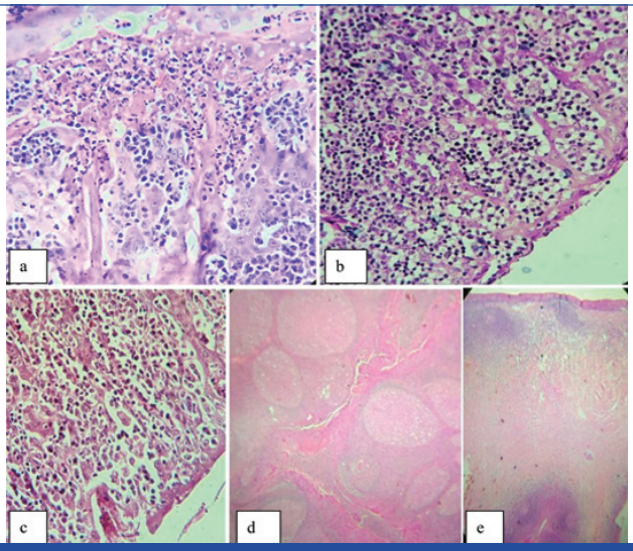
RESULTS

One hundred and forty (140) tonsillar tissue specimens from seventy (70) patients were analysed. *Actinomyces* colonies were detected in 28 patients that formed Group 1 and those without *Actinomyces* (42) were included in Group 2. Clinicopathological features are summarised in [Table/Fig-4]. Both groups were homogenous for gender (p=0.1391) and age (p=0.2261). Presence of Actinomyces across age groups was uniform and statistically insignificant (p=1.000). Of the 28 patients with *Actinomyces*, eight showed a bilateral presence (8×2=16 tonsil specimens) while in the other 20 patients only one-sided tonsil revealed the bacterial colonies (20×1=20 tonsil specimens). The clinical indication for tonsillectomy was recurrent throat infections in 28 cases (28/70=40%) and recurrent throat infections with obstructive symptoms in 42 cases (42/70=60%). The tonsillar specimens showed a relatively uniform gross appearance with grey brown, capsulated lateral surface and irregular, medial crypt surface. Cut surface was tan, homogenous. The mean measurements of the tonsillar specimens in the longitudinal, anteroposterior and transverse dimensions were 2.54±0.61 cm, 1.68±0.5 cm and 0.88±0.38 cm respectively. Of these 36 tonsil specimens harbouring *Actinomyces*, in 17 they were lying superficially and in 19 were present deep within the crypts. Tissue reaction [Table/Fig-1] was seen in 13 of the 36 specimens (36.11%) with more frequent occurrence in bacterial colonies deeply invading the tonsillar crypts (11/19 specimens; 57.89%) as compared to more superficially situated ones (2/17 specimens; 11.76%). A comparison of different microscopic levels of histopathological characteristics as per Reis LG et

al., [Table/Fig-5] between the two groups is shown in [Table/Fig-6] [14]. The number of specimens showing a particular level of microscopic alteration (N) and their percentages (%) in the two groups are depicted. Neutrophilic permeation around the crypt epithelium (was statistically significant between the two groups (p=0.02918) being higher in the Group 1 (with *Actinomyces* colonies) [Table/Fig-5]. Reticulation and plasma cell infiltration around crypts, fibrosis, lymph follicle and germinal center numbers showed no statistically significant differences between the two groups (p>0.05). None of the tonsillar specimens studied showed necrosis.

Clinical findings	Group 1 (With actinomycosis)	Group 2 (Without actinomycosis)	p-value*
Total	28	42	
Sex (M/F)	15/13	15/27	0.1391
Average age (years)	13.6±6.63	11.8±7.19	0.2261
Age ≤20 years	25 (89.29%)	37 (88.1%)	1.000
Age >20 years	3 (10.71%)	5 (11.9%)	

[Table/Fig-4]: Clinicopathological correlation between patients with and without actinomycosis of tonsils.
*Chi-square test and t-test used

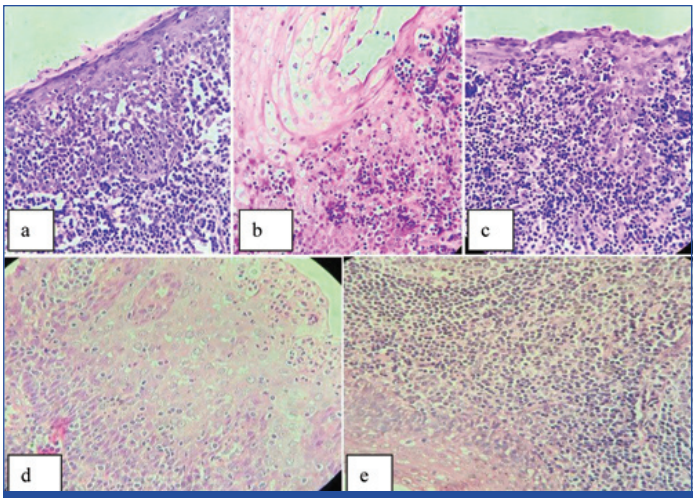


[Table/Fig-5]: Histologic images-Reis LG et al., criteria (H&E).
5a-cryptitis (NEU-CRIPT)(x400); 5b-reticulation around the crypts (RET-CRIPT)(x400); 5c-Plasmacytic infiltration around crypts (INF-PLASM) (x400); 5d-Hyperplasia of tonsillar lymphoid tissue (x40); 5e-Fibrosis and atrophy of lymphoid follicles (x40).

Histo-logical variables	Group	Degree of microscopic alteration					Fisher's exact test p-value
		Level 0 Number (%)	Level 1 Number (%)	Level 2 Number (%)	Level 3 Number (%)	Total	
NEU-CRIPT	1	12 (42.86)	10 (35.71)	6 (21.43)	-	28	0.02918*
	2	30 (71.43)	9 (21.43)	2 (4.76)	1 (2.38)	42	
RET-CRIPT	1	-	3 (10.71)	19 (67.86)	6 (21.43)	28	0.2029
	2	-	12 (28.57)	24 (57.14)	6 (14.29)	42	
INF-Plasm	1	-	6 (21.43)	15 (53.57)	7(25)	28	0.5106
	2	-	13 (30.95)	23 (54.76)	6 (14.29)	42	
Fibrosis	1	12 (42.86)	9 (32.14)	5 (17.86)	2 (7.14)	28	0.7961
	2	14 (33.33)	18 (42.86)	6 (14.29)	4 (9.52)	42	
Necrosis	1	28 (100)	-	-	-	28	1.000
	2	42 (100)	-	-	-	42	
FOL-LINF	1	-	5 (17.86)	21 (75)	2 (7.14)	28	0.2876
	2	-	8 (19.04)	34 (80.95)	-	42	
CEN-Germ	1	-	7 (25)	20 (71.43)	1(3.57)	28	0.5647
	2	-	12 (28.57)	30 (71.43)	-	42	

[Table/Fig-6]: Histopathological analysis (Reis LG et al.,).
*Significant association;
NEU-CRIPT: Permeation by neutrophils in the epithelium around the crypts;
RET-CRIPT: Reticulation around the crypts; INF-PLASM: Plasmacytic infiltration around crypts
FOL-LINF: Number of lymph follicles; CEN-GERM: Number of germinal centers

Statistical comparison between the two groups as regards to the histological variables of Ugras S and Kutluhan A [Table/Fig-7] did not reveal association between any specific histological feature and presence of *Actinomyces* [Table/Fig-8] [14]. Interestingly, acute inflammatory infiltration in the tonsillar surface squamous epithelium and subepithelial (PL) was found with increased frequency in the presence of these bacterial colonies (32% versus 23%).



[Table/Fig-7a-e]: Histologic images- Ugras'criteria (H&E).
7a-The presence of slight-moderate lymphocyte infiltration in the surface epithelium (SMLI) (x400); 7b-Presence of intraepithelial tight aggregates of lymphocytes leading to the defect in the surface epithelium (Ugras's abscess) (x400); 7c-Diffuse lymphocyte infiltration leading to the defect in the surface epithelium (DLI) (x400); 7d-The presence of polymorphonuclear leukocytes in the surface epithelium and in the subepithelial area (PL) (x400); 7e-Increase in the plasma cells number in the subepithelial area and in the interfollicular area (IPCN) (x400).

Histological variables	Group 1 N=28 (%)	Group 2 N=42 (%)	p-value*
The presence of slight-moderate lymphocyte infiltration (SMLI) in the surface epithelium	16 (57.14)	30 (71.4)	0.3288
The presence of abscess leading to the defect in the surface epithelium **(Ugras's Abscess**)	7 (25)	15 (35.71)	0.4945
Diffuse lymphocyte infiltration (DLI) leading to the defect in the surface epithelium	9 (32.14)	16 (38.09)	0.7990
The presence of polymorphonuclear leukocytes (PL) in the surface epithelium and in the subepithelial area	9 (32.14)	10 (23.8)	0.6215
The presence of lymphoid hyperplasia **(LH)**	20 (71.42)	32 (76.19)	0.8670
Increase in the plasma cells number (IPCN) in the subepithelial area and in the interfollicular area	7 (25)	11 (26.19)	1.0000
The presence of fibrosis **(F)**	10 (35.71)	19 (45.23)	0.5859
The presence of atrophy **[A]**	5 (17.85)	8 (19.04)	1.0000

[Table/Fig-8]: Histopathological analysis (Ugras S et al., criteria).
*Chi-square test used

DISCUSSION

The exact role played by *Actinomyces* colonies in adenotonsillar disease is still a debatable topic [2-6]. Most histopathologists ignore the presence of *Actinomyces* colonies in tonsil sections with not much attention given to its location, surrounding tissue reaction. In general, *Actinomyces* species being constituents of normal bacterial flora, are agents of low pathogenicity and require disruption of mucosal barrier, certain predisposing conditions like local trauma, recurrent surgery, irradiation, alcoholism, steroids, HIV, diabetes, chemotherapy to cause disease [16]. Other bacterial species that often are co-pathogens (*Streptococci*, *Haemophilus influenza, Staphylococcus aureus, Moraxella catarrhalis, Mycoplasma*, anaerobes like *Bacteroides fragilis*) to *Actinomyces* species may contribute to spread of infection by inhibiting host defences and reducing local oxygen tension, aided further by the proteolytic enzymes released by the anaerobic Actinomyces colonies [9].

Infection of the oral and cervicofacial region are the most common presentations (55%) of actinomycosis, followed by abdomino-pelvic region (25%), thoraco-pulmonary region (15%) and other rare sites (5%-central nervous system, musculoskeletal system). The source of all these infections is endogenous [7]. Thus, identifying these colonies and verifying any signs of mucosal breach in tissues become clinically important.

Various methods of its detection are macroscopic identification of sulphur granules, histopathology and tissue culture [17]. Although, positive culture studies are the most definite and ideal way of proving their presence, high failure rates (50%) due to overgrowth of other micro-organisms or no growth due to prior antibiotic treatment, inadequate anaerobic conditions, short term incubation make histopathological diagnosis a more feasible option. Characteristic *Actinomyces* colonies showing clumps of basophilic filamentous bacteria in a vague rosette-like configuration with eosinophilic clubs at the periphery (Splendore-Hoeppli phenomena) can be identified with ease in the routine H&E stain and also with other special stains (Gomori's methamine silver/Gram's) on light microscopy [18].

Its incidence in tonsillar specimens has been reported variably in different studies between 1.3 to 57% [2,4,5,19-21]. The present study showed quite a high incidence of *Actinomyces* colonies (40%; 28 out of 70 patients) on microscopic examination. This extreme variability could be due to the different demographic, cultural, environmental factors; variations in the clinical indications of tonsillectomies, pre-operative antibiotic usage and differences in the histopathological laboratory techniques employed [21,22]. Clinicopathological correlation in this study did not reveal any statistically significant gender or age predisposition with the presence of these colonies similar to Gaffney R et al., and Toh ST et al., [4,23]. Some investigators found women to be infected less frequently [24]. Most researchers found statistically higher incidence in adults relative to children [3,6,22,24]. There was no significant difference in mean ages of patients with *Actinomyces* (13.6 years) and those without (11.8 years) in this study.

Most research on tonsillar histopathology focus on crypt architecture (reticulosis, plasma cell infiltration, neutrophilic permeation) and lymphoid follicular architecture. However, Ugras S and Kutluhan A emphasised on the importance of studying the tonsillar surface changes too, which help in differentiating chronic non specific tonsillitis from tonsillar hyperplasia. Therefore, two histological grading modalities which ensured study and comparison of all the tonsillar components were used in the present study [14,15]. Neutrophilic permeation around crypts (cryptitis) showed a significant statistical association (p=0.02918) with the presence of *Actinomyces* colonies. Aydin A et al., in their extensive study on 1820 tonsillar specimens reported common occurrence of cryptitis suggesting it to be a significant histopathological indicator of tonsillar actinomycosis [3]. The statistically significant association between tonsillar cryptitis and tonsillar actinomycosis found in this study, is in agreement with the findings of Aydin A et al., [3]

Tonsillar specimens harbouring *Actinomyces* showed increased frequency of acute inflammatory responses both in the crypts and the surface mucosa. This study did not find evidence to suggest that presence of Actinomyces caused greater degree of tonsillar lymphoid hyperplasia. Some studies found higher presence of *Actinomyces* colonies in tonsillar hypertrophy, few others did not find any direct link to tonsillar hyperplasia [2,24-26]. Commensalism in the true sense is a living relationship between two organisms in which one organism is benefitted without any damage or benefit to the other. Under specific conditions commensal bacteria are able to overcome host defences and exert pathogenic effects. The increased frequency of acute inflammatory tissue responses generated by *actinomyces* colonies both in the superficial mucosal and deep crypt lining of tonsils as seen on histological examination in this study, contradicts their presence as mere innocent by-standers. Once commensal organisms breach host defence barriers (helped by other bacterial co-pathogens) and get access to deeper tissues, the chances of their more wide spread dissemination inside the host are heightened. The most common form of systemic actinomycosis i.e., cervicofacial actinomycosis typically follows mucosal breach due to oral surgeries compounded by poor oral hygiene [8,9]. Therefore, it becomes necessary to identify and treat such early penetrated tissue sites.

Tonsillar cryptitis appears to be an important histological cue in tonsillar *Actinomycosis* and should prompt a more diligent search for these bacterial colonies. It would be helpful if histopathologists while examining tonsillectomy specimens identify the colonies, looks for any evidence of breach of mucosal epithelium and host tissue reaction (deeper location, surrounding suppurative tissue reaction, cryptitis) and report for the presence of any of these. This may have a clinical implication in the form of need for a further, prolonged antibiotic treatment to prevent any future widespread systemic complications in such susceptible individuals.

Limitation(s)

Limited sample size and lack of a clinical follow-up were limitations in this research and a more extensive analysis with a larger study group would be more enlightening and confirmatory.

CONCLUSION(S)

Tonsillar cryptitis was found to be a significant histological finding in tonsillar actinomycosis. Deeply located *Actinomyces* colonies more frequently elicit surrounding suppurative tissue reaction. These acute inflammatory responses generated by the bacterial colonies seem to contradict their role as innocent commensals. It is suggested that whenever *Actinomyces* colonies are found in tonsillectomy specimens, histopathologists look for the evidence of breach of tonsillar epithelial tissues and host tissue reaction around these colonies and mention it in pathology reports of tonsillectomy specimens. This may assist in differentiating mere colonisation from invasion. The need for further antibiotic prophylaxis is left to the clinicians' discretion after taking the clinical presentation into consideration.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
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- Manual Googling: Jul 09, 2021
- iThenticate Software: Aug 04, 2021 (13%)

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- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. No

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