

The Correlation of Micro-organisms between Tonsillar Crypt Culture and Tonsillar Core Culture in Chronic Tonsillitis

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Objectives: This study was undertaken to seek the correlation between tonsillar core and tonsillar crypt cultures and study the incidence of beta- lactamase producing bacteria (BLPB) in chronic tonsillitis patients.

Material and Method: The study was carried out in Department of Otolaryngology from Feb 2000 to Dec 2001. Patients with chronic tonsillitis who underwent tonsillectomy were enrolled, and culture results from tonsillar crypts were compared with tonsillar core.

Results: The tonsil were removed from 61 patients. Age ranging from 2-14 years (n=21) and 15-50 years (N= 40); *H. influenza* (25.2%), *S.aureus* (23.4%), and *S. viridian* (11.3%) were isolated from tonsillar core, while 25.9% and 24% of organisms isolated from tonsillar crypt were *H. influenza* and *S.aureus*, respectively. Correlations between tonsillar core and tonsillar crypt culture were 100% specificity for Group A beta hemolytic streptococcus (GABHS), 86.2% for *H.influenza* and 81.5% for *S.aureus*. Regarding beta-lactamase production, 29.2% of *H.influenza* were beta lactamase producing bacteria (BLPB), while 88.9% of *S.aureus* were BLPB.

Conclusion: The present study demonstrates a high correlation in cultures obtained from tonsillar crypt and tonsillar core. The difference in isolated technique may account for the higher correlation when compared to previous studies. The incidence of beta- lactamase producing bacteria in chronic tonsillitis was high.

Keywords: Tonsillar crypt, Tonsillar core, Bacteriology, Beta-lactamase

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Upper respiratory tract infections are a prime reason for seeking medical attention especially in children. Tonsillitis is one of the common causes of URI, while chronic tonsillitis and recurrent

tonsillitis are still problems in clinical practice in some cases, elective tonsillectomy is used to prevent the recurrence of infection and upper airway obstruction from tonsillar hypertrophy.

The effective treatment of patients with tonsillitis depends on knowledge of the causative organism and their sensitivity pattern. Throat swab

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culture has been used as an integral part in the diagnosis and treatment of tonsillitis. However, obtaining the causative agent from swab culture may not be a good option since the oral cavity is heavily colonized by a variety of bacteria. Previous studies have shown that bacteria on the tonsillar surface might not be the same as the core pathogen in which the etiologic pathogen may be concealed within the tonsillar crypts or parenchyma^(1,2,4,5,6,10).

According to the study of Timon et al⁽¹⁾, the relationship of superficial swab culture compared to the tonsillar core culture was found to be 46.6%, and Surow et al⁽³⁾ report a 43.3% incidence of relation. In contrast, Rosen⁽²⁾ recorded a higher correlation (73%). As the etiologic pathogen might be concealed within the tonsillar crypts, if the authors perform a deep surface swab culture from tonsillar crypt and the results are in good agreement with the bacteriology of the core, then rational treatment of the tonsillitis could be reasonably determined by cultures from deep surface swab.

Timon et al⁽¹⁾ observed several changes in tonsillar microbiology. The respective incidences of *haemophilus influenza* and *staphylococcus aureus* in tonsillar tissue increased from 39% and 6% in 1980 to 62% and 40% in 1989, respectively. Interestingly, the rate of isolation of BLPBs increased similarly. However, Brook et al⁽⁴⁾ reported that the core of the tonsils contained mixed aerobic and anaerobic flora, 85% of which produced beta lactamase. This might be a possible explanation for the failure of penicillin in the treatment of chronic tonsillitis and recurrent pharyngotonsillitis where infection by BLPB's is common, because BLPBs protect not only themselves but also for GABHS⁽⁷⁾.

The aim of the present study was to compare the bacteria isolated from the deep surface of the tonsil with those isolated in the tonsillar core and to study the prevalence of beta - lactamase producing bacteria (BLPB) in the tonsillar core

obtained from chronic tonsillitis patients.

Material and Method

The present study was carried out in the Department of Otolaryngology, Phramongkutklao Hospital, Bangkok, from February 2000 to September 2001. The inclusion criteria of patients were first; patients must be scheduled for elective tonsillectomy due to chronic tonsillitis defined as at least five episodes of inflammation of the tonsil within one year. Second, the age must not be less than 2 years. Exclusion criteria were patients who received antibiotic therapy within 4 weeks before surgery, immunocompromised patients or (and) suspect malignancy.

A complete physical and ENT examination were carried out. Routine investigations including complete blood count and also urine analysis were performed. General anesthesia was administered, before tonsillectomy was performed, the deep surface of the left tonsillar crypts was scraped thoroughly with micro currett, specimen was prepared for culture within 15-30 minutes. After the left tonsil was removed, it was rinsed with sterile normal saline and sectioned with a heated scalpel. An incision was made through the cauterized region by cutting the tonsil in half, a piece of the tonsil core tissue was weighed and homogenized in 10 fold dilutions for colony count, the specimen (tonsillar crypt swab and homogenized tonsil core dilution) were inoculated onto 5% sheep's blood, chocolate and Mac Conkey agar plates for aerobic and facultative organisms. The plates were incubated at 37°C in air (Mac Conkey) or under 5% carbon dioxide (5% sheep's blood and chocolate) and examined at 24 and 48 hours.

For the study of anaerobic pathogens, the materials were plated in duplicate vitamin K1 - enriched Brucella blood agar plates, anaerobic blood agar plates containing Kanamycin and Vancomycin, while anaerobic blood agar plates also containing

phenylethyl alcohol and in enriched thioglycolate broth (Containing hemin and Vitamin K1). The anaerobic plates and thioglycolate were incubated in GasPak jars (BBL-Cockey Ville, MD) and examined at 48 and 96 hours⁽¹⁵⁾.

The isolates were identified by using a standard method. Bacteriology results were reported in percentage of pathogen normal flora⁽⁸⁾. The beta-lactamase activity of all organisms was determined by the chromogenic cephalosporin analog 87/312 method^(9,16).

Statistical Analysis

The result will be presented in percentage of organisms collected from tonsillar core and crypt, both technique of collection will be compared in sensitivity and specificity.

Results

The tonsils were removed from 61 patients, with ages ranging from 2 -14 years (n= 21) and 15 - 50 year (n= 40), 5 specimens were negative of bacteria or grew normal flora with CFU <105 / gm of both tonsillar core and crypt. The most common organisms isolated from tonsillar core were *H.influenza* (25.2%), *S.aureus* (23.4%) and

S.viridans (11.3%) respectively. While, 25.9% and 24% of organisms isolated from the tonsillar crypt were *H.influenza* and *S.aureus* (Table 1).

The organisms frequently isolated from the tonsillar core of children (age<14 years) were *H. influenza* (36.5%), *S. aureus* (29.2%) and *S. viridans* (14.6%). In adults, *S. aureus* (23.1%), *H. influenza* (24.6%) and *K. pneumonia* (15.6%), were among the leading organisms isolated from the tonsillar core. *K. pneumoniae* was isolated only in the adult group (Table 2). Correlation between the tonsillar crypt and tonsillar core were 100% specific for GABHS, 86.2% for *H. influenza* and 81.5% for *S. aureus* (Table 3). Regarding beta-lactamase production, *H. influenza* was 7/31 (22.6%), where as, 24/27 (88.9%) of *S. aureus* was BLBP (Table 4).

Discussion

Of 61 patients with chronic tonsillitis who required tonsillectomy, common pathogens isolated in both children and the adult group were *H. influenza*, *S. aureus*, and GABHS. *K. pneumonia* was found only in adult patients. Interestingly, anaerobic organisms were uncommon when compared with other studies.

Table 1. Comparison of isolated organism in tonsillar core and crypt in chronic tonsillitis

Microorganism	Number of isolated organism	
	In core tonsil (n=61)	in tonsillar crypt (n=61)
- <i>Hemophilus influenza</i>	29 (47.5%)*	27 (44.3%)*
- <i>Staphylococcus aureus</i>	27 (44.3%)*	25 (41.0%)*
- <i>Streptococcus</i> gr.A	11 (18.0%)	12 (19.7%)
- <i>Streptococcus viridans</i>	13 (21.3%)*	9 (14.7%)
- <i>Peptostreptococcus</i>	4 (6.6%)	3 (4.9%)
- <i>Streptococcus</i> gr.D	2 (3.3%)	0
- <i>Klebsiella pneumoniae</i>	10 (16.4%)	8 (13.1%)
- <i>Neisseria</i> spp.	5 (8.2%)	9 (14.7%)
- <i>Bacteroides</i> spp.	4 (6.6%)	4 (6.6%)
- <i>Pseudomonas aeruginosa</i>	3 (4.9%)	3 (4.9%)
- <i>E.coli</i>	2 (3.3%)	2 (3.3%)

*3 leading common isolated pathogens in the presented study

Table 2. Comparison of isolated organisms in tonsillar core culture in children and adult

Microorganism	Children (2-14 years) n=20	Adult (15-50 years) n=41
Aerobes		
Gram positive cocci		
- <i>Staphylococcus aureus</i>	12 (60%)*	15 (36.6%)*
- <i>Streptococcus viridans</i>	6 (30%)*	7 (17.8%)
- <i>Streptococcus</i> gr. A	4 (20%)	7 (17.8%)
- <i>Streptococcus</i> gr. D	0	2 (4.9%)
Gram negative bacilli		
- <i>H. influenza</i>		
β -lactamase positive	3 (15%)	4 (9.7%)
β -lactamase negative	10 (50%)*	12 (29.3%)*
- <i>Klebsiella pneumoniae</i>	0	10 (24.4%)*
- <i>E. coli</i>	0	2 (4.9%)
- <i>Pseudomonas aeruginosa</i>	1 (5 %)	2 (4.9%)
Gram negative cocci		
- <i>Neisseria</i> spp.	2 (10%)	3 (7.3%)
Anaerobes		
Gram negative bacilli		
- <i>Bacteroides</i> spp.	1 (5%)	3 (7.3%)
- <i>Peptostreptococcus</i>	1 (5%)	3 (7.3%)

*3 leading common isolated pathogens in the presented study

Table 3. Correlation of isolated organisms between tonsillar crypt and core cultures

Microorganism	number of organism in core tonsil	Number of organism in both tonsillar crypt And tonsillar core	correlation between tonsillar crypt and core culture (%)
- <i>H. influenza</i>	29	25	86.2
- <i>S. aureus</i>	27	22	81.48
- <i>Streptococcus</i> gr.A	11	11	100
- <i>Streptococcus viridans</i>	13	7	53.8
- <i>K. pneumoniae</i>	10	7	70
- <i>Peptostreptococcus</i>	4	1	25
- <i>Streptococcus</i> gr.D	2	0	0
- <i>Neisseria</i> spp.	5	3	60
- <i>Bacteroides</i> spp.	4	3	75
- <i>P. aeruginosa</i>	3	1	33
- <i>E.coli</i>	2	2	100

Table 4. Demonstrate percentage of beta lactamase producing micro organisms

Microorganism	Children (<14years)	Adult (>14 years)
<i>H. influenza</i>		
BLP positive	3 (23.1%)	4 (33.3%)
BLP negative	10 (76.9%)	12 (66.7%)
<i>S. aureus</i>		
BLP positive	12 (100%)	12 (83%)
BLP negative	0 (0%)	3 (17%)

In 1980, Brook et al discovered that among aerobic organisms, common pathogens of recurrent tonsillitis were GABHS, *S. aureus*, *H. influenza* and among anaerobic organisms, *Bacteroides* spp., *Fusobacterium* spp. and *Peptococcus* spp. were prevalent⁽⁴⁾. Surow et al (1989) reported that anaerobic organisms were uncommon 9/114 of isolates, *S. aureus*, *H. influenza* and GABHS were

among those common micro-organisms in tonsillar core pathogen, but low yield in culture from the surface tonsil, with the largest group were normal flora⁽³⁾.

The surface swab culture did not reflect the presence of pathogen in the tonsillar core according to the study of Surow who found only 33% of isolates from the surface and core tonsil were the same organism, and 14% of isolates in both sites were normal flora⁽³⁾. In the present study, the correlation in percentage of organisms isolated from the tonsillar core and tonsillar crypt was high for common pathogens but low for unusual micro organisms which differ from previous studies^(3,4). The technique used for collection of the specimen for tonsillar surface, namely micro-curettage from tonsillar crypts and core tissue culture may account for these observations. According to the present study, a correlation of the organisms on tonsillar core and tonsil crypt is 100% for GABHS, 86.2% for *H. influenza* and 81.48% for *S. aureus*, while *Bacteroides* spp. is 75%. Brook et al also found 70% of aerobic isolates were recovered in both core and surface specimens, compared with 57% of the anaerobic isolates⁽⁴⁾. Brodsky studied the relationship of surface and core culture in chronic tonsillitis in children reported that 61.5% of aerobic bacteria found in tonsillar core were also identified in the surface of the tonsil⁽¹⁷⁾.

After acute infection, bacteria may harbor in the core leading to chronic infection. Identification of pathogens in tonsillar core and giving an appropriate antibiotic might reduce the number of patients requiring tonsillectomy. If the correlation between tonsillar crypt and tonsillar core isolate is high, tonsillar crypt culture will be useful for determining the appropriate antibiotic for recurrent tonsillitis patients.

Regarding Beta-lactamase producing pathogen (BLPB) in the present study, the number of BLPB among *H. influenza* is still low, 29.2%,

compared with *S. aureus* which is nearly 90%. According to the report by Brook et al, BLPB increased from 1977 to 1993. BLPB were detected in 37 tonsils (74%) during 1977-1978 compared against 47 tonsils (94%) in 1993-1994. All isolates of *S. aureus* and *B. fragilis* groups obtained between 1977 and 1994 produced Beta-lactamase. A similar increase was noted with *H. influenza*, rising from 17% in 1977 to 50% in 1993⁽¹²⁾. Gaffney also showed that *H. influenza* and *S. aureus* were among the leading common bacteria isolated from tonsillar core of both children and adults. The number of specimens containing a beta lactamase producer in children under 7 years old was 43%, 53% for 8-14 years-old, and 39% for patients greater than 15 years⁽¹⁸⁾.

Nevertheless, reproducibility of the high relationship has to be demonstrated. Other parameters such as tonsillar size, the technique used for collection of specimens from crypt of tonsil, might influence the correlation. Would the collection of tonsillar crypt isolated by microcurettage for culture to be useful for the clinician to select the appropriate antibiotics in recurrent tonsillitis patients. Further study in this area is needed.

Conclusion

The present study shows a high correlation between microorganisms cultures from core tonsils and tonsillar crypts with micro curette. It might be beneficial for patients with chronic recurrent tonsillitis; exactly it would reduce number of tonsillectomies.

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การศึกษาความสัมพันธ์ระหว่างเชื้อแบคทีเรียใน tonsillar crypt และ tonsillar core ในผู้ป่วยทอนซิลอักเสบเรื้อรัง

ประสิทธิ์ มหากิจ, กริธา ม่วงทอง, ธนนันท์ สมบุญนา, สุดาลักษณ์ จันทรธาดา

วัตถุประสงค์: การวิจัยนี้เป็นการศึกษาหาความสัมพันธ์ระหว่างผลของการเพาะเชื้อจาก tonsillar core และ tonsillar crypt และศึกษาอุบัติการณ์ของแบคทีเรียที่สร้างเบต้า-แลคตาเมส ในผู้ป่วยที่มีต่อมทอนซิลอักเสบเรื้อรัง

วัสดุและวิธีการ: การศึกษาในผู้ป่วยทอนซิลอักเสบเรื้อรัง ที่มาผ่าตัดทอนซิลที่กองโรค คอ นาสิกกรรม โรงพยาบาลพระมงกุฎเกล้า ระหว่าง มกราคม พ.ศ. 2542 ถึง ธันวาคม พ.ศ. 2544 โดยเปรียบเทียบผลเพาะเชื้อแบคทีเรียที่ได้จาก tonsillar crypt (เก็บโดยวิธี Microcurettage) กับ tonsillar core

ผลการศึกษา: ผู้ป่วยที่ได้รับการผ่าตัดทอนซิลออกจำนวน 61 ราย อายุระหว่าง 2-14 ปี (2 ราย) 15-50 ปี (40 ราย) พบเชื้อ *H. influenza* ร้อยละ 25.2 *S. aureus* ร้อยละ 23.4 *S. viridan* ร้อยละ 11.3 ซึ่งแยกได้จาก tonsillar core ขณะที่พบ *H. influenza* และ *S. aureus* ร้อยละ 25.9 และร้อยละ 24 ตามลำดับ จากการแยกเชื้อจาก tonsillar crypt ความสัมพันธ์ระหว่าง tonsillar core และ tonsillar crypt มีความจำเพาะสำหรับ *Gr. A-B hemolytic streptococcus (GABHS)* ร้อยละ 100 *H. influenza* พบร้อยละ 86.2 และ *S. aureus* สำหรับเชื้อที่สร้าง beta lactamase พบร้อยละ 81.5 *H. influenza* ร้อยละ 29.2 และ *S. aureus* ร้อยละ 88.9 ของเชื้อสามารถสร้างเอนไซม์ดังกล่าว

สรุป: การศึกษานี้พบว่าผลเพาะเชื้อมีความสอดคล้องกันทั้งจาก tonsillar crypt และ tonsillar core เทคนิคการเก็บเพาะเชื้ออาจเป็นปัจจัยที่ทำให้ได้ผลสอดคล้องดีกว่าการศึกษาที่เคยทำมาก่อน
