

Histological and Immunohistochemical Analysis of Special Compartments of Palatin Tonsils in Relation to Tonsillar Diseases

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ABSTRACT

Background: The tonsils are lymphoepithelial tissue, it contains specialized lymphoid functional compartments which include the lymphoid follicles, para-follicular areas, crypt epithelium and high endothelial venules, which together have an essential role in the immunological process. These compartments may be altered histomorphologically throughout life time underneath common pathological condition.

Aim: The aim of the current study is to evaluate special microstructural functional compartment changes as high endothelial venules, lymphoid follicles, interfollicular and connective tissue areas according to histopathological ground of the palatine tonsils.

Methods: one hundred palatine tonsillar samples which were attained from patients suffering from chronic tonsillitis, recurrent tonsillitis and obstructive hypertrophic tonsils were admitted at Al-Jumhuri Teaching Hospital and Al-salaam teaching hospital in Mosul city during the period from February 2018 to February 2019. Age of patients ranged from (2-40) years. Specimens of tissue were directly fixed in 10% formalin then processed. Paraffin sections of 4µm thickness were exposed to routine stain with hematoxylin and eosin, while the studied marker (CD34) was detected by immunohistochemical method using labelled streptavidin-biotin (LSAB/HRP) method.

Results: The high endothelial venules found in the subepithelial compartments as well as with in the reticulated crypt epithelium were characterized by prominent nuclei of the endothelial cells with non-epithelial cells were found on the luminal side. The mean count of high endothelial venules was peak with statistically significant in recurrent tonsillitis and hypertrophic tonsil in both surface epithelium 1.67 ± 0.24 , 0.78 ± 0.22 and crypt epithelium 1.89 ± 0.31 , 0.89 ± 0.20 ($p=0.046$, $p= 0.032$) respectively. However the percentage of follicle area compartment in the tonsillar hypertrophic cases was greater than in other infectious tonsillar diseases (30.33%) respectively. Contradictory, the interfollicular and connective tissue areas reach their maximum in chronic tonsillitis.

Conclusion: The high endothelial venules are dispersed throughout the surface in addition to crypt epithelial. Follicular area percentage in the hypertrophic tonsils is higher than that in the chronic and recurrent tonsillitis group, representing a hyperplastic state of lymphoid cells in the germinal centers. It can also explain the alteration in immune defense mechanism underlying these pathological conditions.

Keywords: high endothelial venules, tonsilectomy, follicular area, tonsillar diseases. , crypt epithelium.

التحليل النسيجي والنسيجي الكيماي المناعي لبعض الاجزاء الخاصة باللوز الحنكية وعلاقتها بأمراض اللوزتين

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الخلاصة

المقدمة : تعتبر اللوزتان من الانسجة اللمفاوية الظهارية , وهي تحتوي على مقصورات وظيفية لمفاوية متخصصة والتي تشمل الجريبات اللمفاوية , المساحة بين الجريبات اللمفاوية , النسيج الضام والاوردة البطانية العالية التي لها دور اساسي في العملية المناعية . قد يتم تغيير هذه المقصورات النسيجية طوال فترة الحياة وتحت تأثير حالات مرضية معينة .

الهدف من الدراسة: تهدف هذه الدراسة الى تقييم التغيرات النسيجية التركيبية الوظيفية للأجزاء الخاصة في اللوزتين الحنكية مثل الأوردة البطانية العالية, الجريبات اللمفاوية , منطقة ما بين الجريبات اللمفاوية والنسيج الضام حسب القاعدة المرضية للوزتين.

طريقة العمل: جمعت العينات وتضمنت اللوز المصابة من المرضى التي أجريت لهم عملية استئصال اللوزتين في مستشفى الجمهوري التعليمي ومستشفى السلام التعليمي في الموصل خلال الفترة من شباط ٢٠١٨ إلى شباط ٢٠١٩ أما بسبب كبر حجمها لأنها تسبب شخيراً أثناء النوم أو الإصابة المتكررة أو المزمناً بالتهاب اللوزتين. قد جمعت مئة عينة من المرضى التي كانت أعمارهم تتراوح ما بين ٢ إلى ٤٠ سنة. بعد جمع العينات حفظت مباشرة في الفورمالين المتعادل بتركيز ١٠% ثم قطعت النماذج بسلك ٤ مايكرون وصبغت بصبغة الهيماتوكسولين ايبوسين إضافة إلى صبغة متخصصة في المناعة النسيجية وصورت المقاطع المنتخبة لتقييم التغيرات النسيجية للأجزاء التركيبية الوظيفية التي سببتها أمراض اللوزتين.

النتائج: بينت هذه الدراسة وجود الأوردة البطانية العالية في النسيج الضام إضافة إلى وجوده في الخبايا والذي يعرف بوجود النواة البارزة للخلايا المكعبة في بطانة الأوعية الدموية. كما بينت الدراسة وجودها بشكل ملحوظ في التهاب اللوزتين المتكرر وفي تضخم اللوز في ظاهرة السطحية 0.78 ± 0.22 , 1.67 ± 0.24 و الخبايا 0.89 ± 0.20 , 1.89 ± 0.31 بفرق معنوي $p=0.032$ أما بالنسبة لمساحة الجريبات اللمفاوية تزداد نسبتها زيادة ملحوظة مع تضخم اللوز أكثر من امراض التهاب اللوزتين (٣٠.٣٣%) وبالعكس أن مساحة منطقة ما بين الجريبات اللمفاوية والنسيج الضام تزداد في التهاب اللوزتين المزمن.

الاستنتاج: لقد لوحظ وجود الأوردة البطانية العالية في النسيج الضام إضافة إلى وجوده في الخبايا بدرجة تميز عالية. بالإضافة الى ازدياد نسبة مساحة الجريبات اللمفاوية في حالات تضخم اللوز بسبب فرط التنسج في الخلايا اللمفاوية في المراكز الجرثومية وهذا يوضح التباين في الوسيلة المناعية الدفاعية في هذه الحالات المرضية.

الكلمات المفتاحية: الأوردة البطانية العالية, استئصال اللوزتين, مساحة الجريبات اللمفاوية , امراض اللوزتين و بطانة الخبايا .

INTRODUCTION

The human palatine tonsils represent flesh collection of lymphatic tissue in the upper part of digestive tract's epithelium¹. They have significant role in immune system of body, in addition they protect the mucosa of alimentary tract against various pathogens².

The palatine tonsil constitute the main lymphoid components in the lymphatic Waldeyer ring³. Histomorphology of the human palatine tonsil shows that the functional compartments of tonsils consist of the lymphoid follicles, parafollicular areas and crypt epithelium which have an essential role in the immune system^{2,4}.

By a process of lymphocyte recirculation, the lymphocytes continuously circulate from blood to lymphoid and other tissues, and back through the lymphatics to the blood to pass the circulation as one of the main actions of the immune system response in the body. Moreover, adhesion of lymphocytes to endothelial capillary is the first step

in lymphocyte immigration from circulation to lymphoid tissue. High endothelial venules (HEVs) is specific postcapillary vascular structures which present only in secondary lymphoid organs. In addition, they serve as a route to pass T and B lymphocytes cells⁵. Furthermore, they are believed to maintain lymphocyte recruitment into chronically inflamed even in non-lymphoid tissues⁶. The endothelial cells of (HEVs) have characteristic shape cells, and because of this, named as high endothelial cells⁷. Light-microscopic examination revealed another characteristic of (HEVs) that is the presence of lymphocytes as a high percentage within their walls⁸. As there are no afferent lymphatic vessels in palatine tonsils⁹ lymphocyte trafficking shows a specific role in disseminate of primed lymphocytes in all functional compartments of the palatine tonsils, this trafficking depends on the presence of high endothelial venules.

The development of HEVs is a prerequisite for the development of germinal center. T-cell homing within germinal centres is required for humoral B-cell responses. As HEVs are essential for this recruitment, they are present in direct proportion to the immunological activity of the reactive lymphoid compartments. Subsequently they influence on progressing, adhesion activation and transmigration of lymphocyte cells¹⁰.

Aim Of Study

The aim of current study is to evaluate special microstructural functional compartment changes as high endothelial venules, lymphoid follicles, interfollicular and connective tissue area in relation to pathological ground of the palatine tonsils and to observe the distribution of high endothelial venules beneath and within the surface and the crypt epithelium.

MATERIALS AND METHODS

This is a prospective cross-section study performed on 100 patients consulting ENT department for tonsillectomy at Al-Jumhuri Teaching Hospital, Al-salaam teaching hospital, Al-Rabea private Hospital in Mosul city during the period from February 2018 to February 2019. Forty four of these patients were female and fifty six patients were male. The age of patients ranged from (2-40) years. Detailed history, including name, age, gender, occupation, history of a sore throat, weakness, chronic fatigue, fever, halitosis, difficult swallowing, loss of appetite joints pain, and snoring, the number attack per year and the duration of illness, were noted. Physical examination signs include tonsillar erythema, swollen soft palate with uvula deviation to contralateral side, enlarged tonsil, edema, cervicular lymphadenopathy, fever, dysphonia, a purulent exudative coating of white patches, drooling and trismus were appreciated. The patients were admitted to hospitals for tonsillectomy are allocated to one of three groups according to clinical examination depending on how many episodes of acute tonsillitis they had been suffering from per year prior from admission also depended on the rate of recurrence and severity of such episodes. First group was those patient with recurrent tonsillitis who defined as seven episodes of tonsillitis in the last twelve

months or five episodes per year in the past two years or three episodes per year for the past three years, accompanied by the symptoms as throat pain and odynophagia, tonsillar exudate / swelling or fever >38. In addition the patients suffered from chronic sore throat, bad breath and persistent tender cervical nodes in other word persistent of symptom more than three months despite of adequate treatment diagnosed as second group (chronic tonsillitis) as well as persistently enlarged tonsils with no sign of infection was measured as hyperatrophic tonsils (third group). Specimens of tissue were fixed immediately in 10% formalin and processed. Paraffin sections of 4- μ m thickness were obtained and stained by hematoxylin and eosin for histological examination. We used trichrome, masson, aniline blue staining to identify connective tissue distribution in palatine tonsils. The studied marker CD34 was detected immunohistochemically using LSAB2/HRP method. CD34 was expressed equally in the capillaries within the surface and crypt epithelium. The vascular endothelium was assessed immunohistochemical representative on formalin fixed paraffin embedded blocks using Monoclonal Mouse Anti Human CD34 class II, clone QBEnd¹⁰. Isotype, IgG1 kappa Number "P28906" (Dako Company, Denmark), stain with labeled streptavidin Biotin detection systems (LSAB /HRP method) for detection of CD34 positive. The positive reaction of CD34 express as brown membrane staining, negative control experiments were conceded without taking the primary antibody.

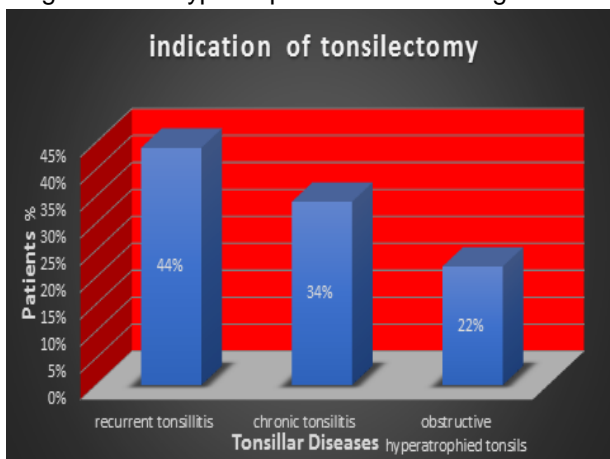
Morphometric Study

Using a color USB 2.0 digital image camera (Scope Image 9.0- China) which was provided with image processing software with light microscope (Olympus, Japan at 100x and 400x magnification power). Quantitative morphometric estimation was carried out on tonsillar sections to determine the numerical areal density of immunopositive cells. Counting of positive CD34 cells was done in 10 randomly chosen high-power fields section, means were calculated and then compared by statistic method. The follicular area was measured/ μ m² by encircling the follicles within field area of 4853147.2 μ m², the summation of follicular areas was multiplied by 100 and divided

on the field area to get the percentage of follicular area and by using the same way, connective tissue area was calculated. As well parafollicular area was calculated by subtracting the summation of follicular and connective tissue area from the whole field area (Iji *et al.*, 2001). The software of the camera was calibrated to all lenses of Microscope-Olympus-CX31 by the aid of 0.01mm stage micrometer (ESM-11 / Japan). The statistical result was considered significant if p value ≤ 0.05 .

RESULT

The patients group included fifty-six males and forty-four females. The mean age of females was 13.31 ± 2.11 years with range 2 – 36 year, while the mean age of male was 10.77 ± 1.71 with range between 2.5 -34 years. In the present study the most common indications for tonsillectomy was recurrent tonsillitis (44%), 34% of patient having chronic tonsillitis and only 22% of the patients were diagnosed as hypertrophied tonsils Graph 1 .



Graph 1 indication of tonsilectomy

A well-organized stratified squamous nonkeratinized epithelium covered the mucosal surface of the palatine tonsil. When the surface epithelium traced towards the crypt it started to lose its organization, and become thinnest gradually. Numerous (HEVs) were present under or within special epithelial compartments. They were recognized by special brown color of cuboidal endothelial cells with prominent nucleus which were surrounded by several lymphocyte cells.

Table 1 shows analysis of the mean count of (HEVs) in surface and crypt epithelium according to pathological ground categories. The results show that the existence of (HEVs) was positive for all of tonsillectomy specimens. Additionally, there was a significant difference in mean count of (HEVs) according to tonsillar diseases except for those of chronic tonsillitis which was statistically non-significant. On the other hand the numbers of (HEVs) is significantly higher in recurrent tonsillitis compared to chronic tonsillitis and hypertrophic diseases in both surface and crypt epithelium ($p=0.046$, $p= 0.032$) as in the (Figures 1, 2, 3) respectively. Although the (HEVs) seen in the surface epithelial compartments in addition to the crypt epithelium but its existence beneath the crypt epithelium is more than that of the surface epithelium with insignificant statistical difference between them.

Table 1 number of high endothelial venules in surface epithelium and crypt epithelium according to pathological ground categories:

Tonsillar diseases	Mean number \pm SD of high endothelial venules / X 100 field		P** - value
	Surface epithelium	Crypt epithelium	
Chronic tonsillitis	1.11 \pm 0.26	1.56 \pm 0.24	0.229
Recurrent tonsillitis	1.67 \pm 0.24*	1.89 \pm 0.31*	0.576
Hypertrophic tonsil	0.78 \pm 0.22*	0.89 \pm 0.20*	0.715
P*-value	0.046	0.032	

* One way ANOVA test & post hoc(Duncan)Test was used.

** Independent t-test was used.

Table 2 analysis of the percentage of the follicular, interfollicular, and connective tissue areas according to histopathological ground categories: This table shows that the percentage of follicle area in the tonsillar hypertrophied group is largest when compared with other infectious tonsillitis groups (30.33%) Figure (4). Also percentage of

interfollicular areas reaches maximum in chronic tonsillitis (74.41%) compared to recurrent tonsillitis and hypertrophic tonsils (72.02%,64.28%) Figure (5) while, patients with chronic tonsillitis have high percentages of connective tissue area (8.32%) than those with recurrent tonsillitis (6.82%) and hypertrophied tonsils(5.39%) Figure (6)

Table 2 the percentage of the follicular, interfollicular, and connective tissue areas according to pathological ground categories.

	% follicular area	% inter follicular area	%Connective Tissue area
Recurrent tonsillitis	21.16	72.02	6.82
Chronic tonsillitis	17.27	74.41	8.32
Hypertrophic tonsils	30.33	64.28	5.39

DISCUSSION

Recurrent tonsillitis is the most common indication to performed tonsillectomy in children¹¹. Specific markers are certainly beneficial to study the distribution of (HEVs) in tissue compartments of palatine tonsils under different pathological conditions, the immunohistochemical technique provides a useful tool to determine the expression of vascular marker (CD34) in endothelial cells of the blood capillary in the human palatine tonsil¹¹. The special lymphoid compartments of palatine tonsils need lymphocyte homing through HEVs for their functions¹². In this study, the reactivity of these compartments was indirectly measured by the number of HEVs. The reticulated crypt epithelium and surface epithelium regarded as functional compartments in the palatine tonsils where the immune reactions occur. The presence of HEVs in the lymphoid tissue beneath the surface epithelium and even in the subepithelial connective tissue indicates that some level of immune reactivity occurs even deep to the thick barrier of surface epithelium¹³. Asma *et al.*,(2008) revealed that the mean number of HEVs beneath the crypt reticulated epithelium was significant more than the same beneath the epithelia of surface (p = 0.000 in each case),

These finding are in conflict with what has been reported in this current study, since HEVs beneath the crypt epithelium was increased insignificantly than they beneath surface epithelium. A nearly similar finding had been reported previously by Indrasingh *et al.*, (2002) who found that the

immune reactions is limited in the surface epithelium of palatine tonsils¹⁴. This indicated that the recognizable level of immune reactivity occurs more in crypt epithelium than in the surface epithelium, which explain by increasing number of HEVs in the crypt epithelium more than in surface epithelium(15). Mal *et al.*, (2008) recommended that the major functions of the reticulated epithelium are to provide a favorable environment for the intimate contact between the effector cells of immune responses, to facilitate direct transport of antigens, to synthesise the secretory component continually and to contain a pool of immunoglobulins. Thus the reticulated epithelium lining the tonsillar crypts represents a specialised compartment, important in the immunological functions of the tonsil as a whole¹⁶. In the current study the number of (HEVs) was significantly higher in recurrent tonsillitis compared to hypertrophied tonsils and chronic tonsillitis, roughly there is no previous study regarding the presence (HEVs) according to diseases of the tonsile but there is general agreement that the (HEVs) can play an important role in the pathogenesis of chronic inflammatory diseases since (HEVs) have been contributed in mechanism for increasing lymphocyte entry into the inflamed tissue, consequently participate in the maintain and amplification of chronic diseases^{12,13,15}. The development of HEVs after prolonged inflammatory stimulus occur in many tissues, particularly the gut and thyroid, such as in inflammatory bowel diseases (Crohn's disease and ulcerative colitis) or the thyroid in autoimmune

thyroiditis (Graves' disease and Hashimoto's thyroiditis)¹⁷. These observations suggest that HEVs could play an important role in the pathogenesis of tonsillar diseases by mediating lymphocyte recruitment to the tonsils

According to the table (2), the percentage of follicle area in the tonsillar hypertrophy group is larger than that in the other groups. Furthermore the percent of interfollicular areas reaches its maximum in chronic tonsillitis (74.41%) compared to recurrent tonsillitis and hypertrophied tonsils (72.02%,64.28%) in addition, these patients have more probability to have high percentage of connective tissue area than those with recurrent tonsillitis and hypertrophied tonsils (8.32%) The current results are consistent with other previous study which have reported that the mean follicle area in the tonsillar hypertrophy group is significantly higher when compared with those in the recurrent tonsillitis group ($P<0.01$)¹⁸. The tonsillar hypertrophy is characterized histologically by an enlargement of follicles, thus representing a hyperplastic disorder of lymphoid cells in the lymphatic follicles. It can explain the difference in etiology or/and immune defense mechanism underlying these illnesses. The study of Avramović *et al.*, confirmed that there is a different mechanism of immune response in tonsils with recurrent tonsillitis compared to hypertrophied tonsils¹⁹. Therefore, the mean follicle area is increased in hypertrophied tonsils compared with recurrent tonsillitis. In addition, Fang *et al.*, indicated that different immune lymphocyte cells morphological subset suggesting different activity of follicular cells and, consequently difference in immune response²⁰. The interfollicular region is characteristic as the subepithelial space between the lymphoid follicles, where its region containing T lymphocyte cells, dendritic cells, macrophages, and high endothelial venules²¹. According to some researches interfollicular area is reduced because of the enlarged lymphoid follicles in tonsillar hypertrophy and it is relatively increased in diseased tonsils. The interfollicular area is reduced because of the enlarged lymphoid follicles in tonsillar hypertrophy as well as it increased to some extent in certain diseases of tonsils²². this is in agreement with some previous work, Saima *et al.*, (2015) who stated that the interfollicular area modification is independent to tonsillar diseases²³.

This recommends that the immunological activity of the palatine tonsil differs in patients with hypertrophic and recurrent tonsillitis, which in practice make a problem concerning the choice of surgical or conservative management¹⁹. Luciana *et al.*, showed no significant difference in the connective tissue area between hypertrophied tonsils and chronic tonsillitis, however they agree with the current study in observing a tendency towards a higher percentage of connective tissue in chronic tonsillitis²⁴. Parenchymal thickening and scarring of connective tissue due to chronic inflammation are one of the basic alterations in diseased tonsils²⁰. However, the fibrosis of the tonsillar tissue due to repeated tonsillitis result in damage of barrier function of the tonsils with a local dysfunction of the immunity, which subsequently caused a persistent infections²⁵, at same time inflammatory process succeed to proliferate and to activate the fibroblastic producing collagen cells in addition to the immune cells to replace the immunologic active tissue with fibrous tissue²⁶. Consequently, regulating fibrogenic processes may be significant therapeutic option in diseases associated with chronic inflammation²⁷.

CONCLUSION

The high endothelial venules expression by CD34 marking are not uniformly distributed throughout the epithelial tonsillar compartments of human in relation to pathological ground. The mean number of (HEVs) increased non-significantly from the surface to the crypt epithelium. Follicular area percentage in the tonsillar hypertrophy group is larger than that in the chronic and recurrent tonsillitis group.

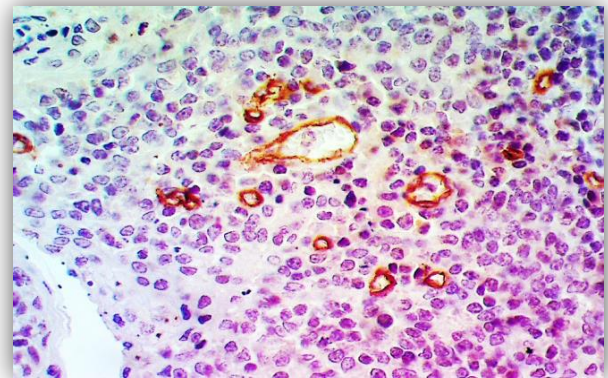


Figure 1: Photomicrograph shows positive staining of endothelial cell CD34 marker in crypt epithelium in patient have recurrent tonsillitis. (IHC, x100)

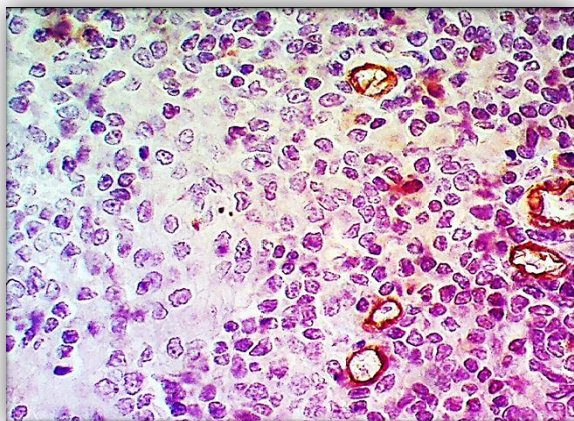


Figure 2: Photomicrograph shows positive staining of endothelial cell CD34 marker showing HEV in patient have chronic tonsil (IHC, X100)

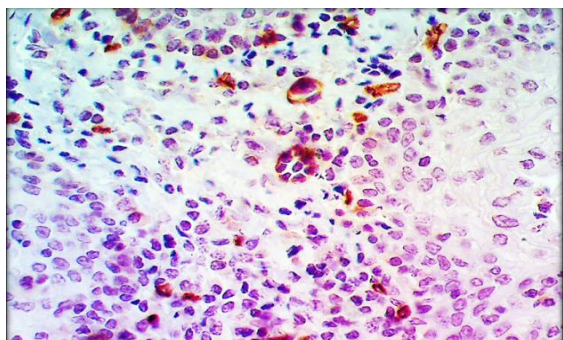


Figure 3: Photomicrograph shows positive staining of endothelial cell CD34 marker in surface epithelium in hypertrophied tonsile (Arrow) Shows HEV (IHC, x100)

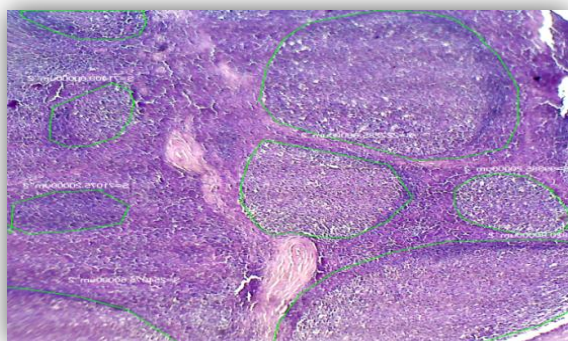


Figure 4: Photomicrograph shows positive staining of endothelial cell CD34 marker in surface epithelium in hypertrophied tonsile (Arrow) Shows HEV (IHC, x100)

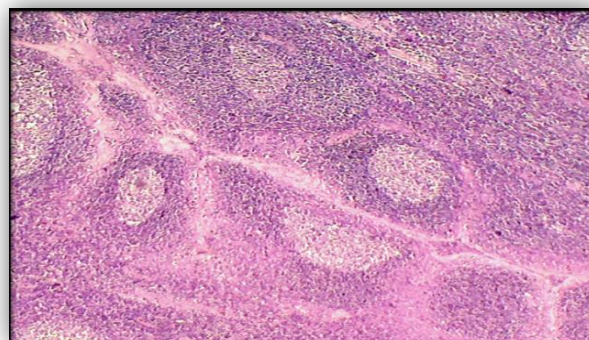


Figure 5: Photomicrographs of palatine tonsils in patient have hypertrophic tonsils showing measurement of follicular, interfollicular, and connective tissue areas:

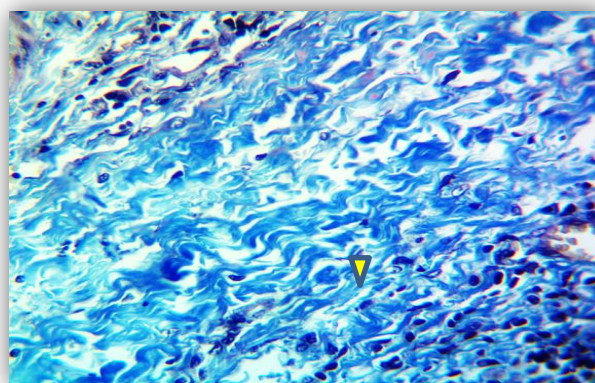


Figure 6: Microscopic section in tonsil: showed positive reaction for connective tissue, Masson's trichrome aniline blue stain 400X

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