

Study of Some Histopathological and Immunological Analysis in Patients with Chronic Tonsillitis

Hetaf Shallal Farhan¹, Ahmed Sami Farhan²

¹Master Student, ²Lecturer, Department of biology Collage of Science, University of Al-Anbar, Iraq

Abstract

Several histopathological and immunological changes that occur in patients with palatine tonsillitis. Blood and tonsil samples were collected from patients with palatine tonsillitis and (healthy peoples) in Ramadi city. The results of histopathological analysis showed the percent of immunopositive cells for the proliferation marker was low while the percent of immunopositive cells for the apoptosis marker was high. Serum was used to know the effect of palatine tonsillitis on some assays CRP, RF, IL-6 and IgM. The results were showed the following: Significant increase in the levels of CRP and RF in patients compared with control group. Significant increase in the levels of IL-6 and IgM compared with control group. We conclude that patients in Ramadi city have several pathological changes in palatine tonsils.

Key Words: *Histopathological Changes, Immunology, Chronic Tonsillitis, Interleukin-6, B-Cell Lymphoma 2.*

Introduction

The human palatine tonsils are lymphoepithelial tissue in the upper part of digestive tract and constitute the main lymphoid components in the lymphatic Waldeyer ring. It contains specialized lymphoid functional compartments which include the lymphoid follicles, parafollicular areas, crypt epithelium, and high endothelial venules, which together have an essential role in the immunological process⁽¹⁾. The tissue consists of B-cell lymphocytes, T-cell lymphocytes and plasma cells which stimulate secretory immunity. B lymphocytes are a group of cells that express clonally diverse cell surface immunoglobulin receptors recognizing specific antigenic epitopes. T-cell derived from thymus and B-cell derived from (bursal or bone marrow)⁽²⁾. T cell is type of lymphocyte, which forms in the thymus gland and plays a central role in the immune response⁽³⁾. They also part of the immune system, because of their location at the throat they can stop germs entering the body through the mouth or the nose. The tonsils also contain a lot of white blood cells, which are responsible for killing germs⁽⁴⁾. There are several functions for tonsils such as provide lymphocytes to the blood and lymph stream, serve as a major defense against bacterial infection⁽⁵⁾. Palatine tonsils are serve as sentinels at the portal

of air and food passage, the crypts in tonsils increases the surface area for contact with strange substances. Tonsils are larger in children and gradually diminish near in adult. They are remove when they themselves become site of disease⁽⁶⁾. The tonsils are creates specific antibodies to a variety of antigens, thereby participates to the normal immunological developmental process⁽⁷⁾. The palatine tonsils include 10% lymphatic cells and constitute approximately 0.2% of all lymphocytes in the adult. Cytologically these are subdivided into small centrocytes (35%), lymphocytes (45%) centroblasts (15%) and plasma cells (2.5%). The centrocytes and centroblasts are germinal center cells. Plasma cells develop from lymphocytes and create immunoglobulins⁽⁸⁾. The aim of the present study to investigate the histopathological changes when the tissue stained with Ki-67, Bcl-2, hematoxylin and eosin stains in addition to measurement the immunological changes in the levels of CRP, RF, IL-6 and IgM.

Methods and Methods

This study was approved by the ethical committee at university of Anbar. The study included thirty patients with chronic tonsillitis attended to the Al-Razi private hospital, where examined under supervision of specialists of ENT and thirty individuals (healthy

peoples) without tonsils problems. Samples of blood and tonsils were taken from all patients during period from August 2020 to September 2020. From all cases, data were collected including age, blood group, treatments given to patient, duration of symptoms for this disease and other parameters through using a short questionnaire. All specimens were fixed in 10% formalin. The tissue were processed to make paraffin blocks. The section were cut at 3-4 micron thickness and stained with ki-67, Bcl-2, hematoxylin and eosin stains to determine the rate of proliferation, apoptosis and other changes in the tissue of the tonsil. Five milliliters of venous blood collected from patients and control groups. The blood were collected in tubes without any Anti-coagulant. These tubes were centrifuged at 3000 rpm for 10 minutes

and then the serum was used for to detect the levels of CRP, RF, IL-6 and IgM.

Results and Discussion

The result of histopathological analysis revealed, the percent of cells for proliferation index was low. This result was agreement with similar study who noticed the cell proliferation occurs less frequently in the interfollicular zone and surface epithelium of tonsils Figure (1) while disagree with other study who found higher proliferative activity in the tonsillar lymphoid nodules. The amount of the Ki-67 antigen indicates the presence or absence of malignant tumors in the palatine tonsils (10). The absence of large quantities of the Ki-67 antigen confirms that no tumors will develop.

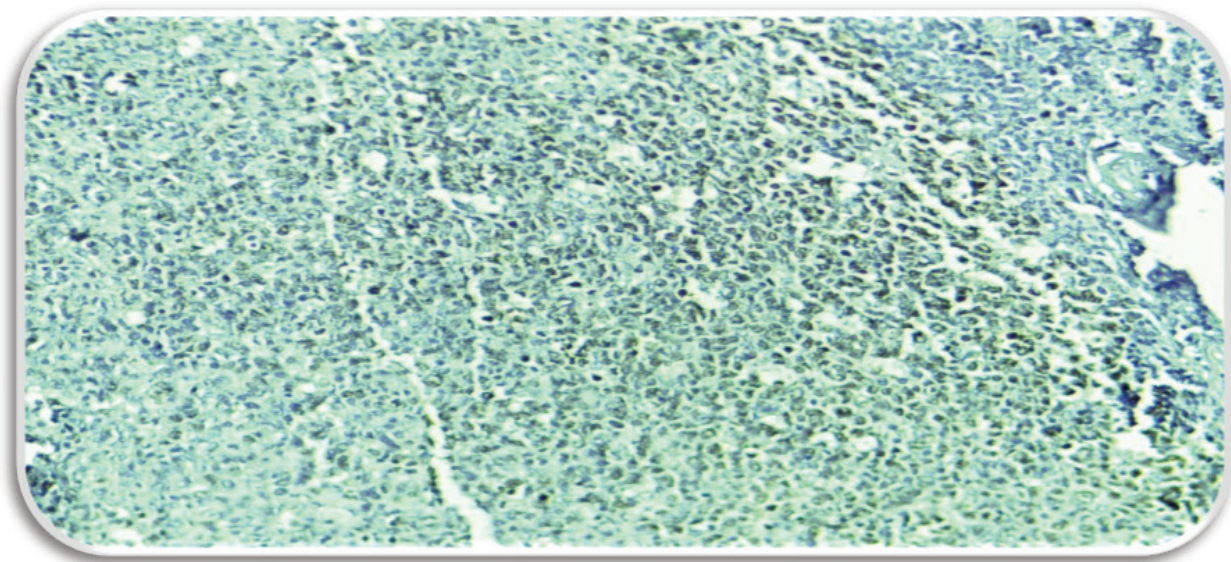


Figure (1): Cross-Section of A palatine Tonsil. 40X. (Ki-67 Stain).

Apoptosis provides an important balance between lymphocyte proliferation in tonsil tissue. The association of apoptosis with tonsillar hypertrophy seem to be age-dependent (11). My result showed the percent of cells for the apoptosis index was high, this result was agreement with previous study who observed, the Bcl-2 is high in

mantle zone cells and absent from most germinal center cells (12) while disagree with other who observed the programmed cell death occurs less frequently in the interfollicular zone and surface epithelium of tonsils (9) **Figure (2)**

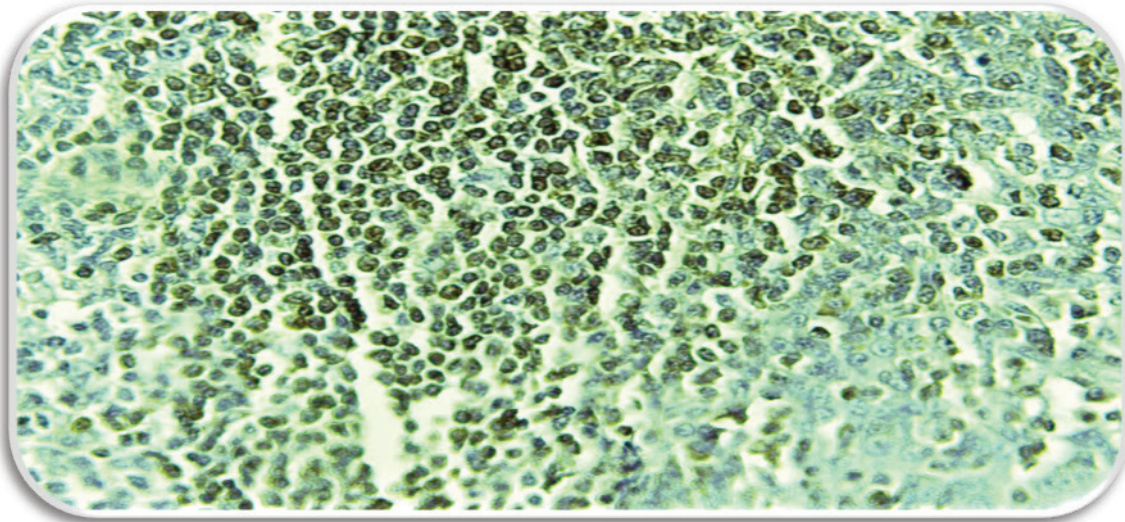


Figure (2): Cross-Section of A palatine Tonsil. 40X. (BCL-2 Stain).

Germinal centers have a dark and light zone surrounded by the mantle zone. The size of germinal center of the lymphoid nodules in the palatine tonsil is an indication to distinguish between the types of tonsillitis **Figure (3)**.

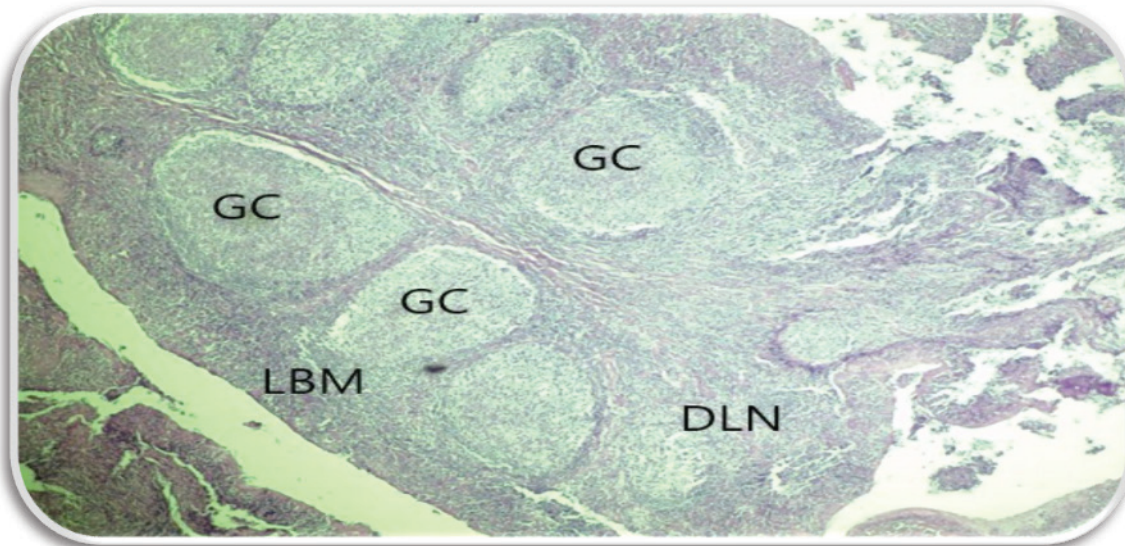


Figure (3): Cross Section of A palatine Tonsil. GC (Germinative Center); DLN (Disappearance of Lymphoid Nodule) and LBM (Loss of Basement Membrane): 10X. (H&E).

Non-keratinized compound squamous epithelium covering the palatine surfaces of the tonsils is subjected to erosion but protected from drying out. As multilayers of the epithelium are usually associated with impermeability of the epithelium layer, it may also be a barrier between exposure to antigens through

the oropharyngeal cavity and the lymph tissue in the tonsils. A study found that epithelial thickness increased or decreased significantly in palatine tonsillitis (14), in addition to lymphocytic infiltration (15) and this is consistent with our study **Figure (4)**.

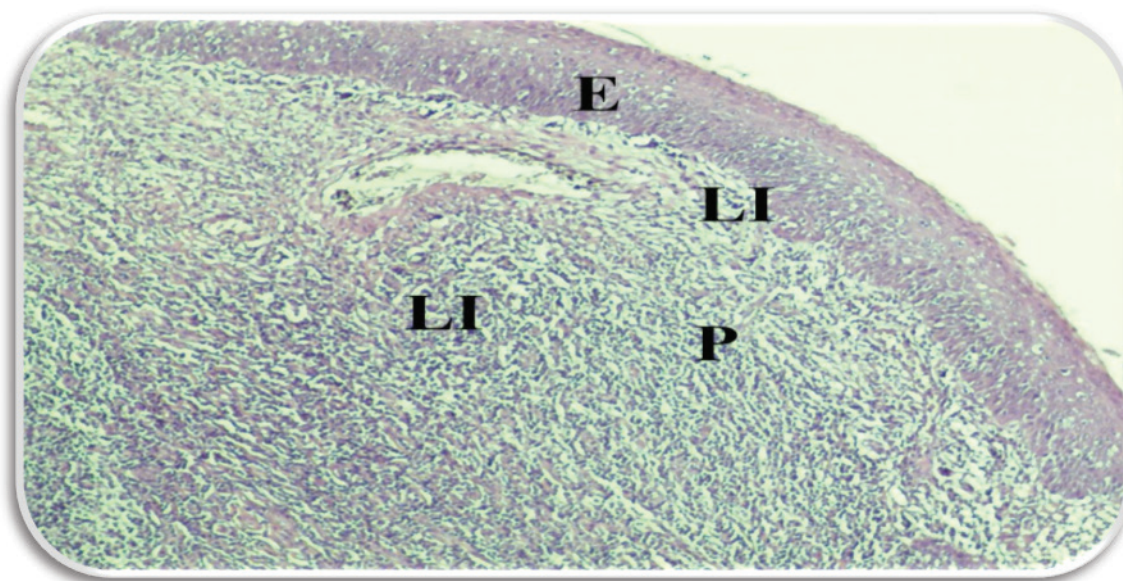


Figure (4): Cross Section of A palatine Tonsil. E (Epithelium); LI (Lymphocytic Infiltration) and P (Parenchyma): 40X. (H&E).

The results of immunological analysis in both patients and control groups are shown. There is a significant increase in the level of CRP in patients when compared with control group. This could indicate that the disease might be developed from bacterial infection which causes chronic inflammation. These results were in agreement with other study who noticed there is significant difference between patients and control group **Table (1)** (16). This result was in disagreement with other study who found there is reduced in C- reactive protein (CRP) value after tonsillectomy which are used as inflammatory markers (17). High level of Rheumatoid factor (RF) is happen because of increasing immune responses to infection, in addition caused by other autoimmune diseases,

such as systemic lupus erythematosus. This result was in agreement with others who found the rheumatoid factor levels are higher in patients when compared with control group **Table (1)** (18). This result was in disagreement with other study who observed tonsillectomy may reduce the risk of RA development, because the surgery may eliminate an essential focus of infection (19). Other study agree with our results considered that tonsillectomy increased the risk of developing RA, but noted that the an earlier tonsillectomy for patients causes significantly lower titers of RF than those who did not, this finding is important because it implicates chronic tonsillar infection cause in the generation of RF (20).

Table (1): Descriptive Analysis for Tests in Both Patients and Control Group.

Case	Tests	Mean	Standard Deviation
Patients	CRP mg/L	15.20	0.837
	RF IU/mL	85.20	2.588
Control	CRP mg/L	4.80	0.837
	RF IU/mL	17.40	1.140

The result is showed the level of IL-6 is high in patients comparison with control .This indicate elevated of IL-6 happen by inflammation, infection, autoimmune disorders. This result was agreement with others who noticed there are increased in the levels of inflammatory cytokines IL-6 in patients compared to control group (21). This result was disagree with others who observed the level of IL-6 levels were reduced after

tonsillectomy (22). The result is revealed increased the level of IgM in patients compared to control group **Table (2)**. High level of IgM happen due to repeated antigenic stimulation. This result was agreed with other studies who noticed IgM is increased after tonsillectomy (23). This result was disagreed with other study who observed the level of IgM is lower in serum of patients compared to control group **Table (2)** (24).

Table (2): Descriptive Analysis for Tests in Both Patients and Control Group.

Case	Tests	Mean	Standard Deviation
Patients	IL-6 pg/mL	56.80	1.924
	IgM g/L	12.60	0.894
Control	IL-6 pg/mL	4.20	0.837
	IgM g/L	2.80	0.837

Conclusion

Bacteria, especially large pus, occur in reducing the body's immunity and producing bodies that work on the occurrence of rheumatoid arthritis or cause failure in kidney . The bacterial infection that affects the tonsils causes some tissue changes in some tissues, especially cartilage, kidney tissues, and the heart.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

References

1. Tabou ZA, AL-Hubaity AY, and Ali EA. Histological and Immunohistochemical Analysis of Special Compartments of Palatine Tonsils in Relation to Tonsillar Diseases. *Ann Coll Med Mosul*. 2019; 41 (2):197-204.
2. Cooper MD, and Alder MN. The evolution of adaptive immune system *Cell*.2006; 124(4):815-822.
3. Alberts B, Johnson A, Lewis J, Raff M, et al. *Molecular Biology of the Cell* 4th edition.2002; Garland Science: New York.
4. Marko J, Verica A, and Aleksandra V. Ultrastructure of the human palatine tonsil and its functional significance. *Romanian Journal of Morphology and Embryology*.2015; 56(2):371-377.
5. Fox S. *Human physiology*. McGraw-Hill Education.2015.
6. Dhingra PL. *Diseases of Ear, Nose, Throat*. 4th ed. New Delhi: Elsevier India Private Ltd.2004; 311-315.
7. Brandtzaeg P. Immunology of tonsils and adenoids: everything the ENT surgeon needs to know. *International Congress Series*. 2003 ; 68(3):387.
8. Komorowska A, Komorowski J, Banasik M, Lewkowicz P, and Tchorzewski H. Cytokines locally produced by lymphocytes removed from the hypertrophic nasopharyngeal and palatine tonsils. *International Journal of Pediatric Otorhinolaryngology*.2005; 69(7): 937-941.
9. Kuceraa T, Pacovab H, Veselyb D, et al. Apoptosis and cell proliferation in chronic tonsillitis and oropharyngeal carcinoma: Role of nitric oxide and cytokines. *Biomedical Papers*.2004;148(2): 225-227.
10. Gultekin SE, Senguven B, Klussmann JP, and

- Dienes HP. p16INK 4a and Ki-67 expression in human papilloma virus-related head and neck mucosal lesions.2015; *Investigation Clinical* 56(1): 47- 59.
11. Onal M, Yılmaz T, Bilgic E, et al . Apoptosis in chronic tonsillitis and tonsillar hypertrophy. *International Journal of Pediatric Otorhinolaryngology*.2015;79(2):191-195.
 12. Krajewski S, Bodrug S, Gascoyne R, et al. Immunohistochemical analysis of Mcl-1 and Bcl-2 proteins in normal and neoplastic lymph nodes. *The American Journal of pathology*.1994;145(3):515-525.
 13. Manthiram K, Correa H, Boyd K, et al. Unique histologic features of tonsils from patients with periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome. *Clinical Rheumatology*.2018; 37(5):1309-1317.
 14. Hafeez A, Khan MY, Minhas LA. A comparative histological study of the surface epithelium and high endothelial venules in the subepithelial compartments of human nasopharyngeal and palatine tonsils. *Journal of the College of Physicians and Surgeons Pakistan*. 2009;19(6):333-337.
 15. Ugras S, and Kutluhan A. Chronic tonsillitis can be diagnosed with histopathologic findings. *European Journal of General Medicine*.2008; 5(2):95-103.
 16. Koo CY, and Eisenhut M. Can inflammatory markers distinguish streptococcal from viral tonsillitis?. *BMJ Journal*.2011; 28(8).
 17. Sahin C, Varim C, and Uyanık M. Use of Neutrophils to Lymphocytes Ratio as an Inflammation Marker in Patients With Chronic Tonsillitis. *National library of medicine*.2016; (259):62-65.
 18. Bamujaly A. ANA, RF and CRP in patients with rheumatic symptoms (SLE and/or RA) in the King Abdul-Aziz Hospital, Jeddah-Saudi Arabia. *Journal of General Practice*.2017 ;5(1):1-4.
 19. Koumantaki Y, Giziaki E, and Kaklamani E. Do appendectomy and tonsillectomy reduce the risk of rheumatoid arthritis?. *Journal of Rheumatology* 1997; 24(6): 1241-1244.
 20. Kordy MMA, Zannoun MAS, and El Ghanam MZ. Assessment of Anti-Streptolysin-O Titer in Healthy School Age Children in El Behira Governorate. *International Journal of Medical Arts*.2020; 2 (3): 625-630.
 21. Deutsch E, Kaufman M, Nisman B, and Barak V. Cytokine evaluation in throat infections. *Annals of Otolaryngology and Laryngology*. 1998;107(8):713-716.
 22. Unal M, Ozturk C, and Gorur K. Effect of Tonsillectomy on Serum Concentrations of Interleukins and TNF- α in Patients with Chronic Tonsillitis. *Original Paper*.2002; 64(4):254-256.
 23. Bitar MA, Rameh C, Ataya NF, Najarian A, et al. Alterations in Humoral Immunity After Partial Versus Total Tonsillectomy: A Pilot Study and Systematic Review of Literature. *Journal of Pediatrics Review*.2016; 4(2):19-30.
 24. Radman M, Ferdousi A H K, and Jalali P. Long-term impacts of tonsillectomy on children's immune functions. *Journal of Family Medicine and Primary Care*.2017; 6(2): 169-170.