

# Qualitative Analysis of Glycogen in Healthy & Inflamed Gingiva

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## Abstract:

**Background:** The aim of present study was to qualitatively determine a relationship exist between glycogen content of gingival epithelium and severity of inflammation.

**Methods:** Sample size consists of 75 cases of healthy and various grades of inflammation of gingiva. The lesions examined in the present study consist of 10 cases of healthy gingiva, 27 cases of mild gingivitis, 17 cases of moderate gingivitis and 21 cases of severe gingivitis. Section was stained with Hematoxyllin & eosin to demonstrate the presence of inflammation and PAS to study the presence and absence of glycogen.

**Results:** In the present study it is found that, as severity of inflammation increases, glycogen content of gingival epithelium also increases proportionately. Maximum content of glycogen is found in the gingival epithelium of severe gingivitis. We also can observe that maximum amount of glycogen is present in spinous layer (75%) followed by granular cell layer (69%), corneum (30%) & basal cell layer (27%).

**Conclusion:** The results showed positive correlation between glycogen and gingivitis. Healthy gingiva contains no glycogen or least amount of glycogen. As the severity of inflammation increases amount glycogen also increases.

**Keywords:** Gingivitis, Glycogen, PAS (Periodic Acid Schiff), Diastase

## Introduction:

Gingiva is that part of oral mucous membrane which extends from the dento-gingival junction to alveolar mucosa and is subject to the friction and pressure of mastication. It is made up of stratified squamous epithelium which may be keratinized or non-keratinized. The epithelium covers a dense lamina propria. The gingiva is limited to outer surface of both jaws by mucogingival junction, which separates it from the alveolar mucosa. Gingivitis (inflammation of gingiva) is the most common condition affecting the oral cavity. Nearly 96% of population is affected by gingivitis. Microorganisms present in the gingival sulcus are responsible for the pathologic changes seen in gingivitis. One of the histochemical changes in the gingiva is the concentration of Glycogen which

varies with the severity of gingivitis. According to study by Weinmann et al<sup>8</sup> (1959) there was an increase in the incidence and concentration of glycogen in proportion to the severity of inflammation. Turskey et al<sup>7</sup> observed an inverse relation between glycogen in the epithelium and surface keratinization. Gingivitis is associated with a few histochemical changes and one of them is concentration of glycogen. The existence of glycogen either in normal un-inflamed gingiva or gingiva affected by disease is highly controversial.

Glycogen is synthesized from glucose by way of glucose-6 phosphate and glucose-1-phosphate. Glycogen is stored in injured cells when their metabolism is impaired. The mechanism by which the glycogen tends to accumulate in the epithelial cells of an inflamed epithelium is due to shift in the balance. Though the circulation is improved due to inflammatory process the energy requirement is lowered because the process of keratinization is inhibited and glucose is stored as glycogen.<sup>3</sup>

## Material And Method

The sample size comprised of 75 interdental papilla of gingiva from adult patients attending the hospital for dental treatment. The gingiva was dried with a sterile gauze piece and clinical conditions were assessed by Loe and Silness gingival index (1963)<sup>4</sup>. The papilla was excised under local anesthesia. The biopsy site was covered by periodontal pack after controlling the bleeding. The sections were washed thoroughly with water and then fixed in formalin for 24 hrs. Washing of tissue thoroughly is important to get accurate results because saliva contains amylase which breaks down glycogen in the tissue present. In this method sections were stained by Hematoxyllin, Eosin and Periodic Acid Schiff (PAS) for glycogen with and without diastase predigestion (Lillie)<sup>5</sup> for glycogen digestion. The sections were incubated in 0.5% diastase solution at 37°C for 30 minutes.

## Histological Analysis

Hematoxyllin and eosin assessed for inflammation (Mayekar<sup>6</sup> 1970) as (a) no inflammation (b) mild inflammation (c) moderate inflammation and (d) severe inflammation. Per-iodic Acid Schiff (PAS) stained sections were used to study the

presence or absence of Glycogen. In the epithelium glycogen was demonstrated in the following manner (Ginwalla<sup>2</sup> 1968) (a) Absence of glycogen (Absence), (b) Glycogen scattered throughout the section (minimum) (c) Few areas densely packed while others showing scattered granules (moderate) and dense accumulation of glycogen spread over the entire section (extensive). In this method Per-iodic Acid Schiff (PAS) stains Glycogen (Magenta) and nuclei (blue) or (Blue-Black) but H & E stains Nuclei (blue) & cytoplasm (Pink).

## Result

A total of 75 cases of healthy and various grades of inflammation of gingiva were studied in the present study. The lesions examined in the present study consisted of 10 cases of healthy gingiva, 27 cases of mild gingivitis, 17 cases of moderate gingivitis and 21 cases of severe gingivitis (Table I, II). Hematoxyllin and eosin was used to demonstrate the presence of inflammation to divide the lesions into 4 categories (Mayekar<sup>6</sup>) as Grade 'O' (No inflammation), Grade '1' (mild gingivitis), Grade '2' (moderate gingivitis) and Grade '3' (severe gingivitis). Per-iodic Acid Schiff (PAS) stained sections were used to study the presence or absence of glycogen. In the epithelium glycogen was demonstrated in absence, minimum, moderate and extensive form (Ginwalla<sup>2</sup>) 1968.

The observation in present study shows 10 cases of normal gingiva which were biopsied and stained with PAS. Out of these 7 showed complete absences of glycogen 2 showed minimum amount of glycogen and 1 showed moderate amount of glycogen in the epithelium (Table IV). 27 biopsies of mild gingivitis were taken out of which none showed the presence of glycogen, 16 showed the minimum amount of glycogen, 8 showed moderate amount and 3 showed extensive amount of glycogen (Table VI). In moderate gingivitis 17 cases were studied out of these 11 biopsies showed moderate amount of glycogen, 3 showed extensive amount of glycogen, 2 were showing minimum amount of glycogen and 1 biopsy showed complete absence of glycogen (Table VI). In severe gingivitis 21 biopsies were examined. There were 12 biopsies which show extensive glycogen, 5 shows moderate and 4 biopsies show minimum

glycogen (Table VI).

**Table-I : Distribution of Cases**

Condition	Number of Cases	Percentage
Normal gingiva	10	13.33
Mild gingivitis	27	36
Moderate gingivitis	17	22.7
Severe gingivitis	21	28

**Table-II : Cases of Healthy Gingiva**

Sample No.	Amt. of Glycogen	Layers of Epithelium			
		Basal	Spinous	Granular	Corneum
H1	---	-	-	-	-
H2	---	-	-	-	-
H3	---	-	-	-	-
H4	++	-	-	-	-
H5	++	-	-	-	-
H6	++	-	-	-	-
H7	---	-	-	-	-
H8	---	-	-	-	-
H9	---	-	-	-	-
H10	---	-	-	-	-

-- Absence of glycogen, /= Presence of glycogen

**Table-III : Cases of Mild Gingivitis**

S. No.	Sample No.	Amount of Glycogen	Layers of Epithelium			
			Basal	Spinous	Granular	Corneum
1	M1	---	-	-	-	-
2	M2	---	-	-	-	-
3	M3	---	-	-	-	-
4	M4	---	-	-	-	-
5	M5	---	-	-	-	-
6	M6	---	-	-	-	-
7	M7	---	-	-	-	-
8	M8	---	-	-	-	-
9	M9	---	-	-	-	-
10	M10	---	-	-	-	-
11	M11	---	-	-	-	-
12	M12	---	-	-	-	-
13	M13	---	-	-	-	-
14	M14	---	-	-	-	-
15	M15	---	-	-	-	-
16	M16	---	-	-	-	-
17	M17	---	-	-	-	-
18	M18	---	-	-	-	-
19	M19	---	-	-	-	-
20	M20	---	-	-	-	-
21	M21	---	-	-	-	-
22	M22	---	-	-	-	-
23	M23	---	-	-	-	-
24	M24	---	-	-	-	-
25	M25	---	-	-	-	-
26	M26	---	-	-	-	-
27	M27	---	-	-	-	-

-- Absence of glycogen / = Presence of glycogen

From tables (II, III, IV, V & VI) it is evident that there is a positive correlation between severity of inflammation of gingiva and the concentration of glycogen in the gingival epithelium. The amount of glycogen is least or there is a complete absence of glycogen in the healthy gingiva. (Table II).

**Table-IV : Cases of Moderate Gingivitis**

S. No.	Sample No.	Amount of Glycogen	Layers of Epithelium			
			Basal	Spinous	Granular	Corneum
1	M01	---	-	-	-	-
2	M02	---	-	-	-	-
3	M03	---	-	-	-	-
4	M04	---	-	-	-	-
5	M05	---	-	-	-	-
6	M06	---	-	-	-	-
7	M07	---	-	-	-	-
8	M08	---	-	-	-	-
9	M09	---	-	-	-	-
10	M010	---	-	-	-	-
11	M011	---	-	-	-	-
12	M012	---	-	-	-	-
13	M013	---	-	-	-	-
14	M014	---	-	-	-	-
15	M015	---	-	-	-	-
16	M016	---	-	-	-	-
17	M017	---	-	-	-	-

-- Absence of glycogen, /= Presence of glycogen

As the severity of inflammation increases the glycogen content of gingival epithelium also increases proportionately (Table III, IV & V), maximum concentration of glycogen is found in the gingival epithelium of severe gingivitis (Table V). Also we can observe maximum amount of glycogen is present in spinous layer (77%) followed by granular cell layer (69%) corneum (30%) & basal cell layer (27%) as in (Table VII).

**Table-V : Cases of Severe Gingivitis**

S.No.	Sample No.	Amount of Glycogen	Layers of Epithelium			
			Basal	Spinous	Granular	Corneum
1	S1	---	-	-	-	-
2	S2	---	-	-	-	-
3	S3	---	-	-	-	-
4	S4	---	-	-	-	-
5	S5	---	-	-	-	-
6	S6	---	-	-	-	-
7	S7	---	-	-	-	-
8	S8	---	-	-	-	-
9	S9	---	-	-	-	-
10	S10	---	-	-	-	-
11	S11	---	-	-	-	-
12	S12	---	-	-	-	-
13	S13	---	-	-	-	-
14	S14	---	-	-	-	-
15	S15	---	-	-	-	-
16	S16	---	-	-	-	-
17	S17	---	-	-	-	-
18	S18	---	-	-	-	-
19	S19	---	-	-	-	-
20	S20	---	-	-	-	-
21	S21	---	-	-	-	-

-- Absence of glycogen, /= Presence of glycogen  
**Table-VI : Distribution of Glycogen in the epithelium in Various Gingival Conditions**

Inflammatory infiltration	Total Number	Complete absence	Minimum	Moderate	Extensive
Grade 0	10	7 (70%)	2 (20)	1 (10)	-
Grade 1	27	-	16 (59)	8 (30)	3 (11)
Grade 2	17	1 (6)	3 (18)	11 (64)	2 (12)
Grade 3	21	-	4 (19)	5 (24)	12 (57)

-- Absence of glycogen, /= Presence of glycogen  
**Table-VII : Relation between Glycogen and the Region of Epithelium**

Amount of Glycogen	No. of Cases	Region of the Epithelium			
		Basal	Spinous	Granular	Corneum
Minimum	25	7 (28)	16 (64)	10 (40)	7 (28)
Moderate	24	8 (33)	20 (83)	22 (92)	8 (33)
Extensive	16	12 (75)	14 (87)	13 (81)	15 (94)
Total	65	27 (32)	50 (77)	45 (69)	30 (46)

-- Absence of glycogen, /= Presence of glycogen  
**Discussion**

The inflammation of gingiva (gingivitis) is so common condition in the oral cavity. Plaque plays an important role in the development of gingivitis. Gingivitis is the most common form of gingival disease. Inflammation is almost always present in all forms of gingival disease. The role of inflammation in gingival disease varies in 3 ways:

1. Inflammation may be primary and only pathologic changes may occur.
2. Inflammation may be secondary feature, superimposed on systemically caused gingival disease.
3. Inflammation may be the precipitating factor responsible for clinical changes in patient with systemic conditions.

The most common type of gingival disease is the simple inflammatory involvement caused by bacterial plaque attached to the tooth surface. This type of gingivitis called chronic marginal gingivitis or simple gingivitis may remain stationary for indefinite periods of time or destroy the supporting structures (Periodontitis). Pathological changes in gingivitis are associated with presence of microorganism in the gingival sulcus. In addition to changes caused by organism in gingivitis a few histochemical changes take place like the concentration of glycogen in the epithelium. Different methods have been used for demonstration of glycogen. Glycogen can also be demonstrated by means of silver complexes by different ways.<sup>1</sup> However, common method used is Periodic Acid Schiff reaction which is used in the present study and is the most reliable and an easy

method. In this method 2 slides were prepared to confirm the presence of glycogen. One slide was directly stained with PAS while other was treated with diastase for half on hour at 37°C. 2. Sections were then compared. The part that disappears after diastase treatment but visible in the section stained directly by PAS is glycogen. Glycogen appeared as Magenta Red. Hence no glycogen was observed in clinically normal gingiva while in inflammatory gingival it was comparatively more. The results indicate that there is a positive correlation between glycogen content of gingival epithelium and grades of gingival inflammation observed clinically and with degree of inflammation in the tissues. The association between glycogen and inflammation can be explained on the basis that whenever injury is inflicted upon the epithelial cells, changes are produced in connective tissue which influences the nutrition or physiology of epithelium resulting in the deposition of glycogen. Thus a hypothesis may be formulated that glycogen is formed from the excess glucose produced in the adjacent inflamed tissue by gluconeogenesis.

**Summary And Conclusion:**

A total of 75 biopsies were studied for glycogen estimation of the normal and inflamed gingiva. Each section was stained by Hematoxyllin and eosin for histologic grading of biopsy specimen. Sections were also stained by PAS directly and others after diastase predigestion.

From the study conducted following conclusion were drawn

1. There is a relationship between gingivitis and glycogen.
2. There is a positive correlation between glycogen and gingivitis.
3. Healthy gingiva contains no glycogen or least amount of glycogen.
4. As the severity of inflammation increases the amount of glycogen also increases proportionally.
5. Maximum amount of glycogen is present in the severely inflamed gingiva.
6. Demonstration of glycogen can be used as histologic criteria for gingivitis.
7. It also can be used as a diagnostic tool.

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