BULLETIN OF STOMATOLOGY AND MAXILLOFACIAL SURGERY
Volume 22, Issue 1

DOI: 10.58240/1829006X-2025.22.1-120

A HISTOLOGICAL, HISTOCHEMICAL AND IMMUNOCYTOCHEMICAL ANALYSIS OF SYNDROMES WITH GINGIVAL ENLARGEMENT

Enjy Bassim [1], Heba Gamaleldin [2], Sahar Sabry [3], Shymaa Hamza[4], Mona Wali[5]
[1]PhD. Candidate at Oral & maxillofacial pathology department, Faculty of Dentistry, Cairo University, Al Saraya, Old Cairo, Cairo Governorate, 4240310. E-mail: enjy.bassim@dentistry.cu.edu.eg
[2]Researcher at the Oro-dental Genetics Department, Human Genetics and Genome Research Institute, National Research Center, Cairo, Egypt, 33 El Buhouth St, Ad Doqi, Dokki, Cairo Governorate, 12622
E-mail: Heba_mostapha85@hotmail.com
[3]Associate professor at Department of Biochemical Genetics Human Genetics and Genome Research Institute, National Research Centre (NRC), Cairo, Egypt, 33 El Buhouth St, Ad Doqi, Dokki, Cairo Governorate, 12622.
E-mail:sas_pharmacienne@yahoo.com
[4] Lecturer of Oral & maxillofacial pathology, Faculty of Dentistry, Cairo University, Al Saraya, Old Cairo, Cairo Governorate,4240310.E-mail:shymaa.hamza@dentistry.cu.edu.eg
[5] Professor of oral & maxillofacial pathology, Faculty of Dentistry, Cairo University, Al Saraya, Old Cairo, Cairo Governorate,4240310.E-mail:mona.wali@dentistry.cu.edu.eg
Corresponding Author: **Enjy Bassim** PhD. Candidate at Oral & maxillofacial pathology department, Faculty of Dentistry, Cairo University, Al Saraya, Old Cairo, Cairo Governorate, 4240310.
E-mail: enjy.bassim@dentistry.cu.edu.eg
Received: ***Received:*** *Dec 17. 2025;* ***Accepted:*** *Jan 26. 2026;* ***Published:*** *Jan. 31, 2026*

Abstract

Background: A syndrome is caused by a mutation in the DNA sequence. The diagnosis of syndromes is relying mainly on clinical and molecular analysis. Molecular techniques are not available in every institute and not affordable to everyone. Therefore, the need for simple and affordable diagnostic substitutes for these conditions is mandatory. Syndromes with gingival enlargement are diverse although being rare. GCF collection and analysis could aid in the diagnosis of such cases. Besides, tissue biopsy examination could facilitate reaching the final diagnosis in association with clinical findings. The rationale of this study is the use of simple and feasible techniques for the diagnosis of syndromes with gingival enlargement. So, the hypothesis of this study states that if the H& E staining, histochemical staining, and ELISA analysis of certain molecules show superiority or equality in the sensitivity and specificity in the diagnosis of such syndromes, they could be used as a more available and cheaper way of diagnosis.

Material and methods: Total number of 37 cases were involved in the study, among which 12 syndromic patients and 25 cases of control cases. Samples of GCF were collected by using periopapers and analyzed using ELISA kits for IL-8, TNF- α, MMP-2 and GAGs. Gingival tissue biopsies were collected and stained with H&E, AB, PAS, MT and VG stains then examined under the microscope

Conclusions: H&E staining could be considered a valuable tool for the preliminary histopathological diagnosis of PLS, ERS, and JHF, owing to their special microscopic features alongside with unique clinical features. However, histochemical staining did not significantly contribute to diagnostic clarity due to variability in their expression. TNF-**α** showed the highest sensitivity, specificity, and diagnostic accuracy among the markers highlighting its potential as a supportive diagnostic

Keywords: Syndromes; Molecular Diagnosis; Gingival Enlargement; Crevicular Fluid; ELISA; Tissue Biopsy; Special Stain

INTRODUCTION

Syndromes are conditions having multiple manifestations that are always repeated together. Each syndrome is linked to specific genetic mutation responsible for their unique clinical presentation. The maxillofacial and oro-dental areas are amongst the regions that often demonstrate several features of syndromes [1].

In the head and neck area, the gingival tissue, is a commonly affected structure. Particularly manifested in the form of gingival enlargement. Whether syndromic or non-syndromic, it is a common symptom that represents a diagnostic challenge for oral and maxillofacial specialists [2].

Gingival inflammation, often resulting from poor oral hygiene, is the most common cause of gingival enlargement. Additionally, accumulation of certain molecular components may contribute to gingival hyperplasia. Also, fibrotic tissue proliferation is a well-established factor causing gingival enlargement [3].

In oro-dental practice, determining the accurate reason of enlarged gingiva is crucial for proper treatment. Being manifested in many syndromes, reflects that total eradication of the condition may be difficult [4]. This further highlights the importance of identifying the cause, since clinical examination alone, isn't sufficient for accurate diagnosis.

Among the most commonly known syndromes that involve gingival hyperplasia as a part of their clinical presentation is Papillon-Lefevre Syndrome (PLS). PLS is an autosomal recessive (AR) syndrome that is characterized by inflammatory gingival enlargement. The underlying cause of inflammation is defective neutrophilic chemotaxis and activation due to loss-of-function mutation in the cathepsin C gene [5].

Severe periodontitis is the hallmark of PLS patients at the time of deciduous and permanent teeth eruption. These patients experience complete loss of deciduous teeth at the age of 5 years and permanent teeth at the age of 15 years. Although periodontal disease is aggressive in PLS patients, they show complete resolution of inflammation during the edentulous period [6].

Besides oral manifestations, dermatological signs are also noted. Palmo-plantar keratosis is observed in almost all cases of PLS. Keratosis at the elbows and knees, hyperhidrosis, nail dystrophy, and multiple pyogenic infections have been documented in several cases of PLS [7,8].

On the other hand, Enamel Renal Syndrome (ERS) is characterized by a mutation in the FAM20A gene with an AR mode of inheritance. Amelogenesis Imperfecta (AI) and nephrocalcinosis are the main clinical features. Periodontitis and gingival fibromatosis are occasionally detected, to which gingival enlargement in these patients could be attributed [9]. The combination of Cone–Rod Dystrophy (CRD) and AI is the hallmark of Jalili Syndrome (JS). Although different phenotypes of JS have been recently discussed, all reported cases are linked to mutations in the CNNM4 gene responsible for metal transport with AR mode of inheritance [10]. AI is a consistent feature in JS cases, with or without anterior open bite (AOB), and may be linked to fluoride levels in drinking water [11].

Winchester Syndrome (WS) is another AR inherited syndrome with gingival enlargement; together with, hereditary bone osteolysis and coarse facial features. A proposed genetic mutation in matrix metalloproteinase-2 (MMP-2) gene have been raised, which can lead to impairment of fibroblast function [12]. Among the manifestations are hypertrichosis, cataracts and multiple bony joint contractures that may be misdiagnosed as rheumatoid arthritis [13].

Another AR bone osteolysis disease with joint movement limitations is Juvenile Hyaline Fibromatosis (JHF). The main driving mutation of this syndrome is in the Anthrax toxin receptor 2 (ANTXR2/CMG2) gene, that in turn, disrupts collagen metabolism and causes most of the clinical manifestations [14]. Gingival hyperplasia is well noted in almost all cases, with evident gingival overgrowths covering most of the teeth surfaces [15].

Mucolipidosis (MLs) is AR type of lysosomal storage disease with a defect in N-acetylglucosamine-1-phosphotransferase, which is responsible for the phosphorylation of mannose to mannose-6-phosphate that transports more than 70 lysosomal hydrolases. The clinical manifestations of MLs are diverse. Oral manifestations mainly include gingival hyperplasia and macroglossia [16]. Other features like, skeletal abnormalities, cardiomyopathy, hepatosplenomegaly, and neurodegeneration are also reported [17].

The gold standard in diagnosing all these syndromes is molecular detection of their specific genetic mutation. Although being definitive, it is considered expensive and not readily available in all institutes [18]. Among the widely used molecular methods are Polymerase Chain Reaction (PCR) and DNA sequencing [19].

Since, histopathological examination is the benchmark of tissue biopsy assessment [3], therefore, syndromic enlarged gingiva can be thoroughly analyzed using conventional Hematoxylin and Eosin (H&E) stain. This technique has the advent of being lower in cost and is more readily available. Correlating the histopathological findings with the pathogenesis that led to gingival enlargement can aid in diagnosis.

Moreover, histochemical detection of different molecules in gingival tissue could be a possible reproducible method for identifying the reason behind gingival enlargement and, in turn, confirm the diagnosis [20]. Tissue hyperplasia could be due to accumulation of collagen, mucopolysaccharides and glycogen [4, 16]. The

detection of these structures using special histochemical staining could be beneficial in the final diagnosis.

Among the histochemical stains that can detect collagen are Masson Trichrome (MT) and Van Gieson (VG) stains. While MT stains collagen fibers in blue to green color, VG stains the collagen bundles in pink color differentiating them from other surrounding structures [21, 22]. Both stains could be highly valuable in demonstrating the extent of fibromatosis that could be the cause of gingival enlargement in some syndromic cases.

The use of Periodic Acid Schiff (PAS) stains polysaccharides such as glycogen giving magenta color [23]. Therefore, easy to highlight structures like basement membrane and determine its thickness. Alcian Blue (AB) is another stain, used to distinguish mucopolysaccharides and acidic mucins by staining them blue [24].

Beside microscopic examination of gingival tissue, collection of gingival crevicular fluid (GCF) could be used as an adjunctive tool for interpretation of what's going on inside syndromic oral cavity using Enzyme Linked Immunosorbent Assay (ELISA). Although using GCF in syndromic diagnosis is limited, it is well documented in literature that it can aid in the diagnosis of different periodontal diseases [25].

Interlukin-8 (IL-8) and Tumor Necrosis Factor- α (TNF- α) are considered pro-inflammatory cytokines that show altered expression in the GCF of the patients with gingival inflammatory diseases [26]. Therefore, their alteration in GCF could be a valuable diagnostic point. Besides, MMP-2 as an extracellular matrix collagenase enzyme [27] could show altered levels in the GCF of different syndromic patients.

Glycosaminoglycans (GAGs) build-up in the extracellular matrix of tissues in Mucopolysaccharidosis and MLs [28, 29]. The levels of such molecules may be altered in the GCF of the affected patients. Their detection could aid in the diagnosis together with other diagnostic methods.

Due to the exaggerated cost and complexity of the conventional genetic mapping in diagnosing these syndromes, testing other simple, less complicated alternatives can be in the favor of many patients especially in populations with less socioeconomic status and limited resources. In this study, collective use of basic diagnostic techniques such as H&E, histochemical and GCF examination is suggested to identify the cause of gingival enlargement of these syndromes and hence aid in the diagnostic process.

The rationale of the study is to explore simple, cost-effective, and feasible diagnostic techniques for syndromes with gingival enlargement. The hypothesis is that if H&E, histochemical staining, and ELISA analysis of specific molecular markers demonstrate comparable or superior sensitivity and specificity to existing diagnostic methods, they could serve as more accessible and affordable alternatives for diagnosis.

MATERIAL AND METHODS

Material

This was a prospective ex vivo study conducted over a period of 18 months from the study's initiation. Syndromic cases presenting with gingival enlargement were recruited from the Oro-Dental Genetics Clinic at the National Research Centre in Egypt. Each case underwent an initial clinical diagnosis, and a detailed pedigree was constructed. The suspected diagnoses were subsequently confirmed through molecular analysis to detect the relevant genetic mutations.

The **GCF** collection and gingival tissue biopsy procedures were done at the Oro-dental Genetics Clinic of the National Research Center in Egypt. Biopsied tissues were preserved in 10% formalin specimen cups. **GCF** was collected using specialized Perio-Papers then preserved in eppendorf tubes for proper handling.

Kits of ELISA for IL-8, TNF-α, MMP-2, and GAGs were purchased from Sunlong Biotech through Biotech Serve for Chemicals and Laboratories, Giza, Egypt with *Cat. No. (E0089Hu, E0082Hu, E0904Hu, E1409Hu)* respectively. The ELISA analyses were conducted at the Biochemical Genetics Department, Institute of Human Genetics and Genome Research, National Research Centre, Egypt. Gingival tissue biopsies were processed, stained, and microscopically examined at the Oral and Maxillofacial Pathology Department, Dental Research and Supplies Unit, Faculty of Dentistry, Cairo University.

Eligibility criteria for participants:

- Attendance at the Oro-Dental Genetics Clinic of the National Research Centre in Cairo, Egypt, within 18 months from the start of the study.
- Confirmation of a syndromic diagnosis through molecular genetic testing.
- Clinical presentation of gingival enlargement.

Methods

Study population:

This study protocol was ethically approved by the Research Ethical Committee at the Faculty of Dentistry, Cairo University, and was conducted in accordance with the principles of the Declaration of Helsinki.

A total of 37 participants, were enrolled in the study, including 12 syndromic patients and 25 healthy controls. The syndromic group consisted of cases of 5 PLS, 2

cases of ERS, 2 cases of JS, 1 case of WS, 1 case JHF, and 1 case of MLS. However, Control samples were collected from pediatric patients aged 6 to 16 years, matching the age range of the syndromic group, during their routine dental visits.

Samples from syndromic patients were obtained during follow-up sessions at the Oro-Dental Genetics Clinic of the National Research Centre in Cairo. Informed consent was obtained from the legal guardians of all participants involved in the study **(Fig. 1).**

المركز القومي للبحوث
لجنة أخلاقيات البحوث العلمية

نموذج الموافقة المستنيرة
لاخذ عينة بيولوجية لاجراء التحاليل الوراثية

الاسم: ..... ولي الأمر: .....
السن: ..... صلة القرابة: .....
العنوان: ..... التليفون: .....

الهدف من أخذ العينة :

اجراء التحاليل البيولوجية والجينية اللازمة للمريض واجراء المزيد من الابحاث مستقبلا مما يفيد حالة المريض وباقي المرضى.

اقرار صاحب العينة او ولي الامر

1. فهمت كل المعلومات الخاصة باستخدام العينة الخاصة بي في اجراء ابحاث وعلمت ان مشاركتي حرة وتطوعية
2. اوافق على تمكين فريق العمل من جميع المعلومات الشخصية والجذور العائلية المتعلقة بمشروع البحث واخذ عينة لاجراء كل التحاليل البيولوجية والجينية اللازمة.
3. كذلك اوافق ان تحفظ عينة (الحمض النووي) الخاصة بي ويمكن اجراء المزيدمن الابحاث عليها مستقبلا مما يفيد حالتي ويفيد باقي المرضى.
4. علمت ان كل المعلومات المدونة سيتم جمعها وتحليلها ومعالجتها اليا في اطار السرية التامة.
5. عقد النشر العلمي لا تحتوي البيانات على اي معلومات يمكن منها تحديد هوية المشارك الى شخصي وشريطة حماية حرمة الحياة الشخصية وسرية البيانات والعينات البيولوجية.

- وضح لي الطبيب المشرف كل المعلومات المتعلقة باستخدام العينة الخاصة بي في اجراء البحث واعلم ان مشاركتي حرة وتطوعية.

توقيع صاحب العينة او ولي الامر:

اقرار الطبيب المشرف على اخذ العينات

اتعهد بالحفاظ على سرية المعلومات الخاصة بالشخص محل البحث
توقيع الطبيب المشرف: .....

اسم ورقم تليفون المسئول عند وجود اي استفسار من صاحب العينة :

- تم اجازة صيغة هذا النموذج من لجنة اخلاقيات البحوث العلمية بالمركز القومي للبحوث بتاريخ / /

Figure1. The form of taken informed consent

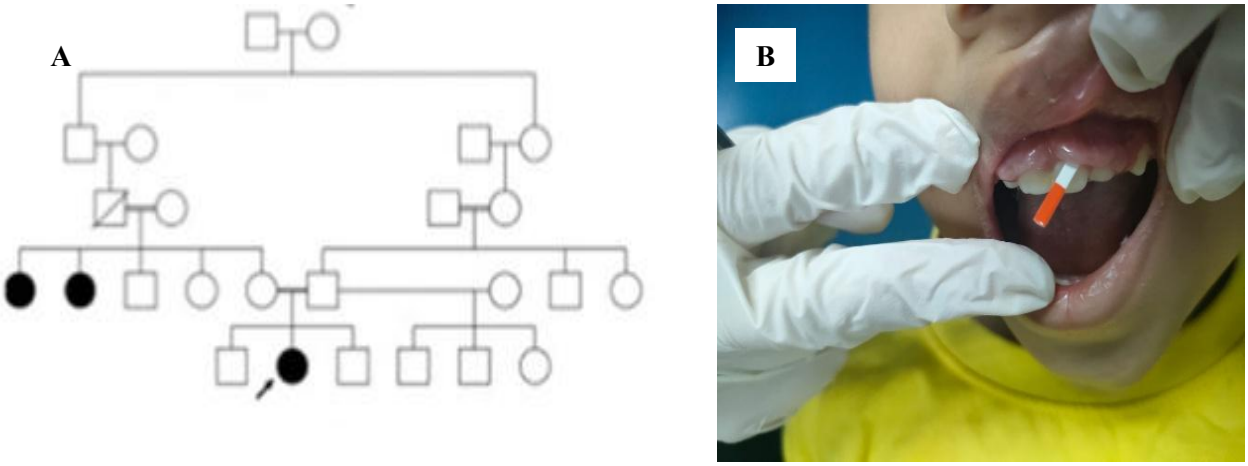


Figure 2. A- Family pedigree, **B-** GCF collection by periopaper

Collection of GCF

GCF collection was done using Gingival Fluid Collection Strips (periopapers) purchased from **ORAFLOW, Smithtown, NK 11787**. **GCF** was collected by inserting the periopapers in the gingival crevice, then preserved in Eppendorf tube **(Fig. 2 -B).** In syndromic cases, sample collection was performed from areas exhibiting clinically evident gingival enlargement.

Analysis of GCF:

The levels of **(IL-8, TNF-α, MMP-2 and GAGs)** were analyzed for both syndromic and control groups by extracting the GCF collected in periopapers from each case and using **ELISA** kits. The ELISA kits were used following the manufacturer's instructions [30]. Results were expressed in either mg/mL or ng/mL.

Collection of tissue biopsies

Gingival tissue biopsies were obtained from both control and syndromic groups, then preserved in 10% formalin specimen cups. Gingival tissue biopsies in syndromic cases were carefully taken from sites showing the greatest degree of clinical enlargement, to ensure representative sampling for histopathological analysis.

H&E and histochemical staining of tissue samples

The biopsied tissues were stained using routine protocols for **H&E**, **AB, PAS**, **MT and VG** stains [31, 32, 33, 34]. The stained sections were then examined under ordinary light microscope.

Statistical Analysis

Numerical data were assessed for normality by evaluating data distribution and applying the Kolmogorov–Smirnov and Shapiro–Wilk tests. All variables demonstrated a normal (parametric) distribution. Accordingly, data were presented as mean and standard deviation (SD) values. Comparisons between the two groups were conducted using Student's *t*-test.

Receiver Operating Characteristic **(ROC)** curve analysis was performed to evaluate the diagnostic accuracy of the tested biomarkers in relation to the gold standard. Pairwise comparisons between different markers were conducted using the *z*-test. A significance level of $P \leq 0.05$ was adopted for all statistical tests.

Statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA). ROC curve analysis was performed using MedCalc® Statistical Software, version 19.5.1 (MedCalc Software Ltd., Ostend, Belgium; https://www.medcalc.org; 2020).

RESULTS

H&E and histochemical stains results:

1.PLS H&E and histochemical stains results:

Papillon Lefevre Syndrome (PLS) (Fig.3 A) tissue specimens revealed intense inflammation **(Fig.3-B, C)** and some destruction of the covering epithelium **(Fig.3- D) (Table 1).**

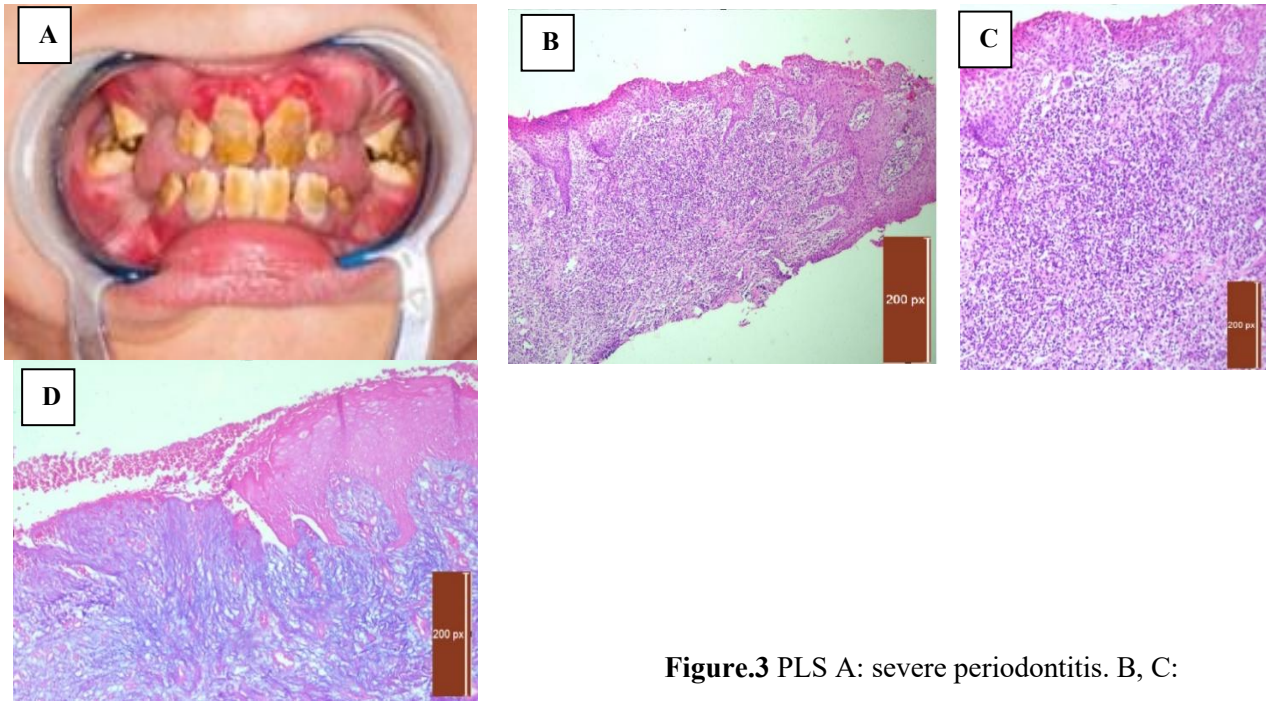


Figure.3 PLS A: severe periodontitis. B, C:

However, no inflammatory infiltrate was noted in the case of teeth clearance with deposition of collagenous stroma instead **(Fig.4-A)**. In one case, the lower one third of the epithelium showed features of dysplasia. Where, bulbous rete-ridges, areas of budding, expanding proliferative epithelial compartments and keratin pearl formation attempt were observed **(Fig.4- B, C) (Table 1).**

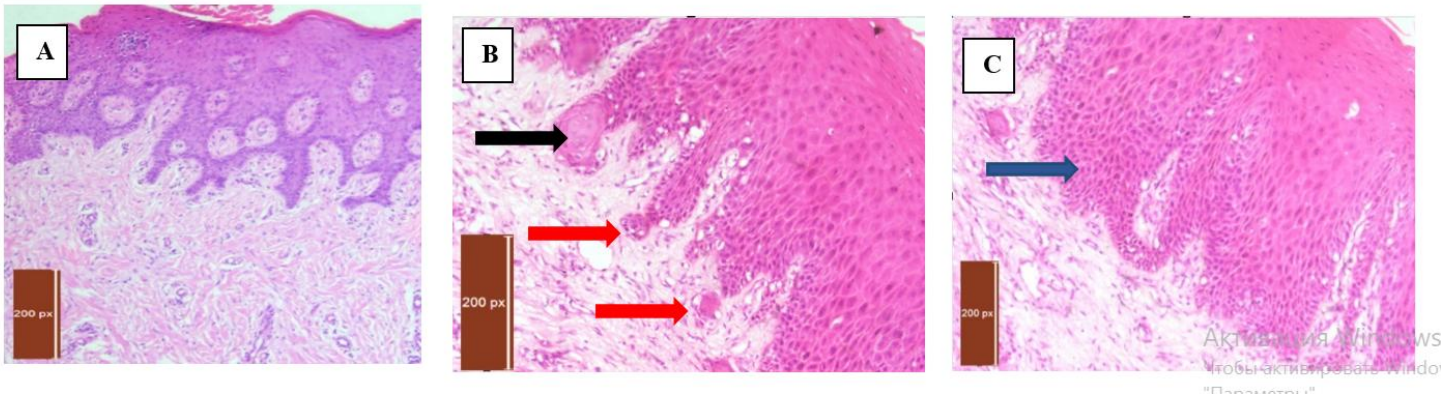


Figure 4 PLS A: H&E section showing collagenous stroma devoid of inflammation. **B, C:** H&E sections revealing basilar hyperplasia, premature keratinization **(black arrow)**, budding **(red arrows)** and expanding proliferative epithelial compartments **(blue arrow).**

The histochemical examination of **PLS** revealed positive reaction with **AB, PAS, MT** and **VG** stains **(Fig.5-A, B, C, D) (Table 2).**

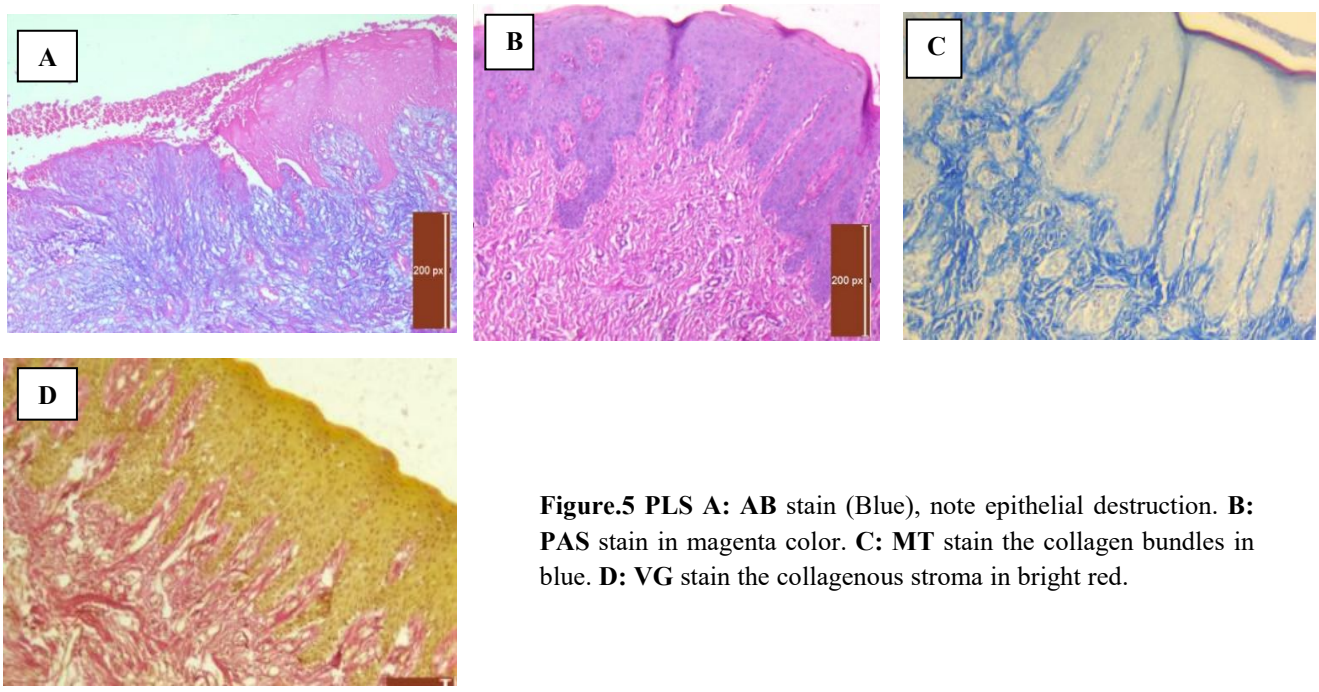


Figure.5 PLS A: AB stain (Blue), note epithelial destruction. **B: PAS** stain in magenta color. **C: MT** stain the collagen bundles in blue. **D: VG** stain the collagenous stroma in bright red.

2 ERS H&E and histochemical stains results:

Enamel Renal syndrome (ERS) (Fig.6 A) tissue specimen revealed epithelial hyperplasia **(Fig.6 B).** Occasional epithelial destruction and intense inflammatory cell infiltration were also noted **(Fig.5 B, C)**. Moreover, calcification with lamellar configuration was evident in both cases **(Fig.6 D, E) (Table 1).**

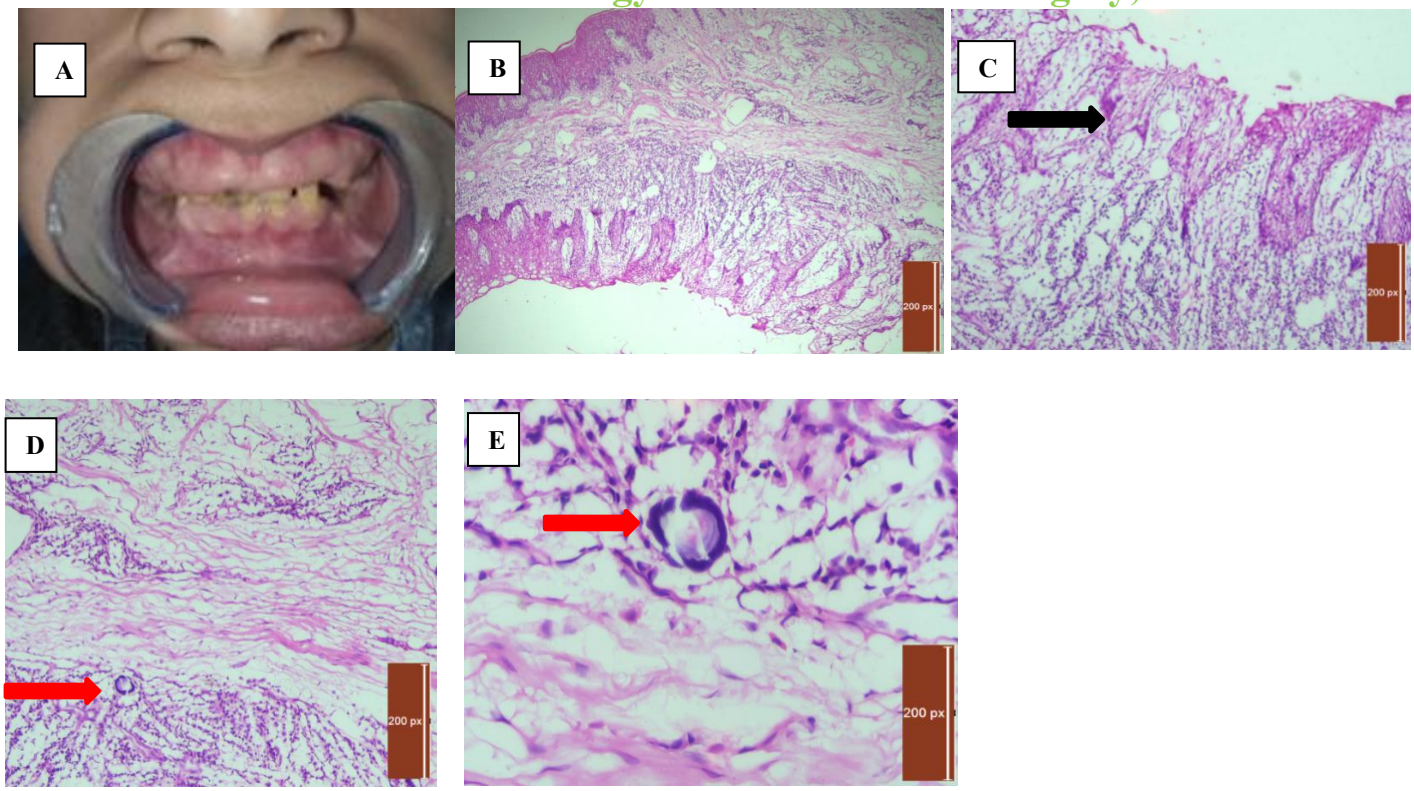


Figure 6 ERS A: Gingival hyperplasia. **B: H&E** section showing epithelial hyperplasia and stromal inflammatory cell infiltrate. **C: H&E** section showing epithelial destruction and heavy inflammatory cell infiltrate **(black arrow). D: H&E** section showing some stromal inflammatory cells and a calcific mass **(red arrow). E: H&E** section showing lamellar calcification in the stroma **(red arrow).**

ERS cases showed negative reaction for **AB (Fig.7-A).** On the other hand, **PAS** stain was positive in the connective tissue stroma and strongly expressed in basement membrane area **(Fig.6- B).** Both **MT** and **VG** stains were positively expressed staining stromal collagen fibers **(Fig.7-C, D) (Table 2).** Epithelial vacuolization was noted in many sections **(Fig.6-A, B, C, D).**

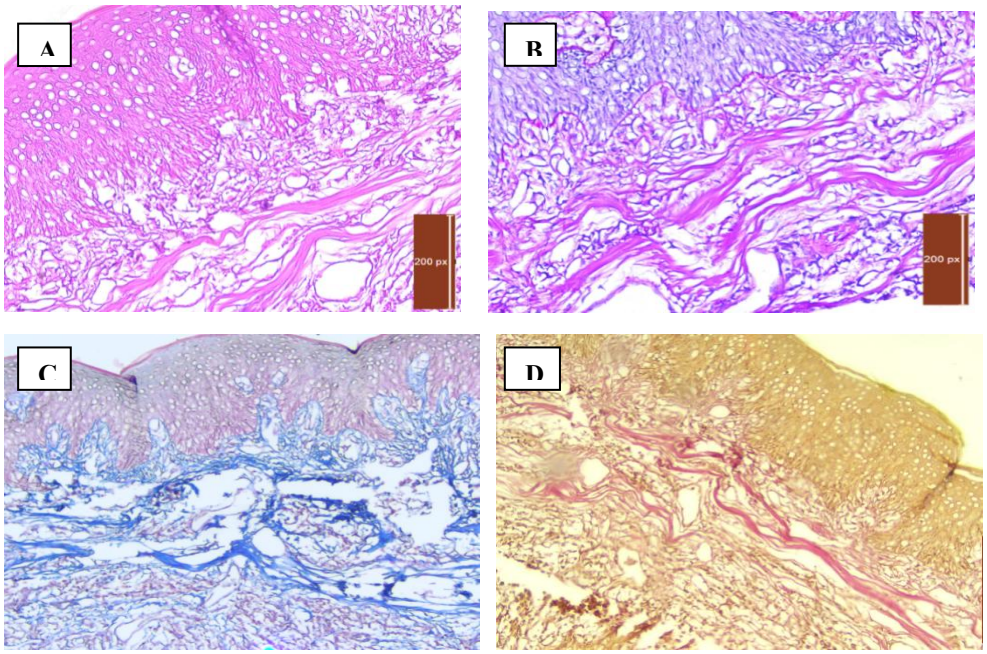
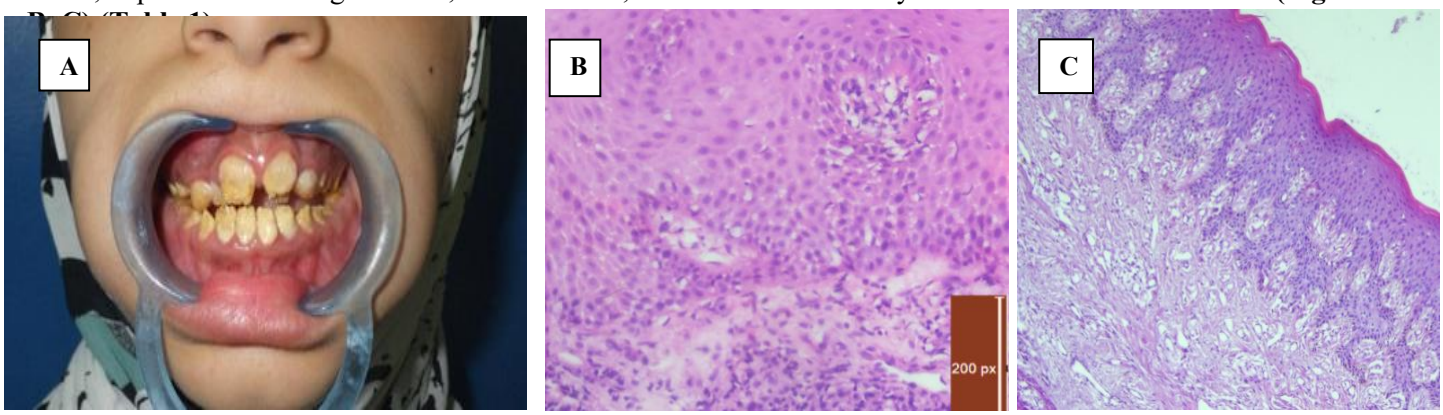


Figure 7. ERS A:AB negative stain, **B: PAS** stain in magenta color, **C: MT** stain in the form of blue collagen bundles, **D: VG** stain collagen content in bright

3 JS H&E and histochemical stains results:

For **Jalili Syndrome (JS) (Fig.8 A)**, epithelial hyperplasia was seen in both cases **(Fig.8 B, C)**. In the connective tissue, deposition of collagen fibers, blood vessels, and mild inflammatory cell infiltrate were also noted **(Fig.8-**



Figur 8. JS A: swollen gingiva. **B, C: H&E** section showing epithelial hyperplasia and stromal inflammation (**B**).

Although, **AB** was negatively expressed in one case **(Fig.9- A)**, the other one showed little expression only under the epithelium **(Fig.9- B) (Table 2). PAS** reaction was scattered between inflammatory cells, however it was intense at the basement membrane area which showed some thickening **(Fig.9- C).** Both **MT** and **VG** were evidently positive in the connective tissue stroma illustrating the heavily collagenous nature **(Fig.9- D, E) (Table 2).**

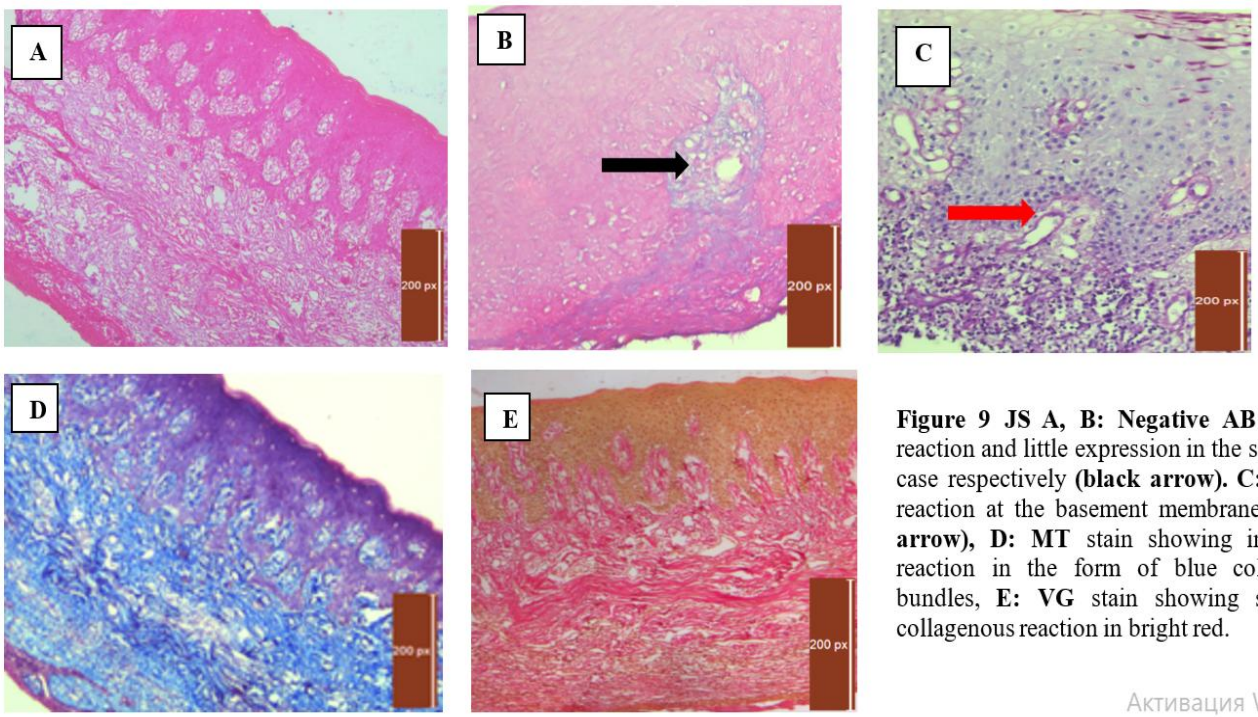


Figure 9 JS A, B: Negative AB stain reaction and little expression in the second case respectively **(black arrow). C: PAS** reaction at the basement membrane **(red arrow), D: MT** stain showing intense reaction in the form of blue collagen bundles, **E: VG** stain showing strong collagenous reaction in bright red.

4.WS H&E and histochemical stains results:

Winchester syndrome's (WS) (Fig.10-A) gingival tissue demonstrated epithelial hyperplasia with moderate lymphocytic infiltration **(Fig.10-B).** Areas of epithelium affected by inflammation were where the inflammatory infiltrate was concentrated **(Fig.10-C) (Table 1).**

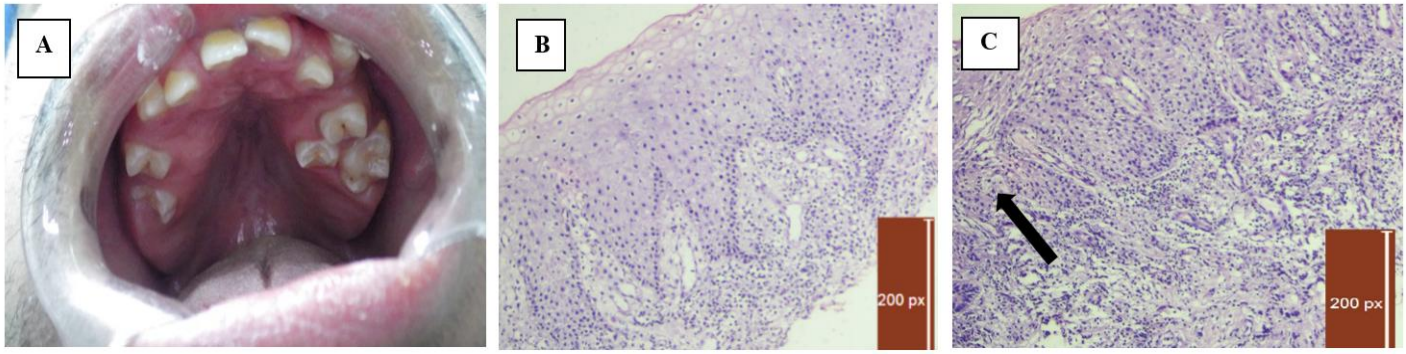


Figure 10. WS A: hyperplastic tissue surrounding misaligned teeth **B:** H&E section showing hyperplastic epithelium and inflammatory cell infiltrate, **C: H&E** section showing intense inflammation and partial destruction of covering epithelium **(black arrow)**.

While **AB** displayed negativity throughout the specimen **(Fig.11- A)**, **PAS** positivity was limited to the collagenous stroma between the inflammatory cell infiltrate **(Fig.11- B)**. Weak **PAS** staining was noted at the basement membrane close to areas of partial epithelial destruction **(Fig.11- B).** There was slight positivity of both **MT** and **VG** stains confined also to the areas of collagen in between the inflammatory cell infiltrate **(Fig.11- C, D) (Table 2).**

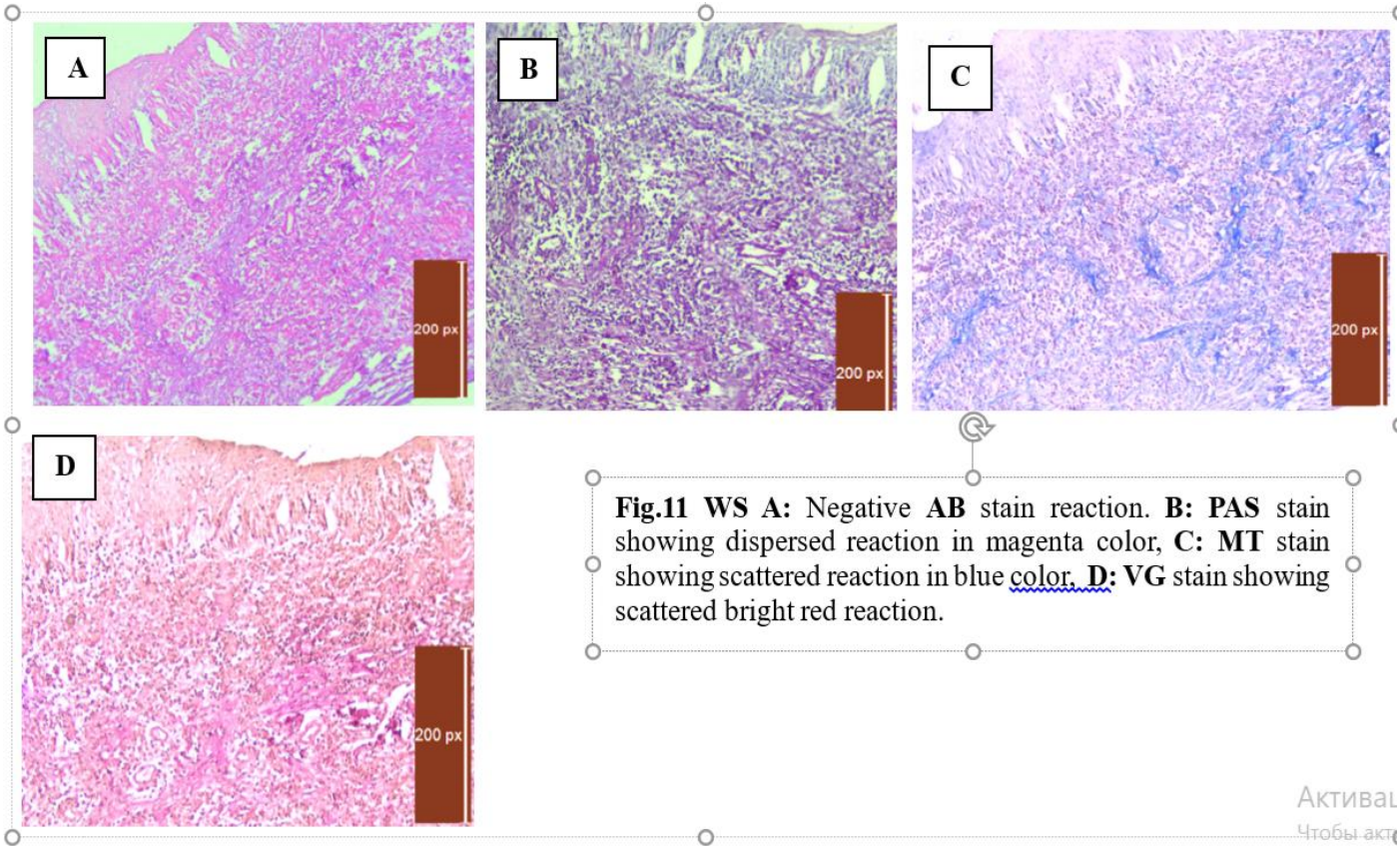


Fig.11 WS A: Negative **AB** stain reaction. **B: PAS** stain showing dispersed reaction in magenta color, **C: MT** stain showing scattered reaction in blue color, **D: VG** stain showing scattered bright red reaction.

5 JHF H&E and histochemical stains results:

Examination of **Juvenile Hyaline Fibromatosis (JHF) (Fig.12-A)** revealed marked hyalinization of the connective tissue stroma**.** The surface epithelium also showed hyperplasia, while the basement membrane demonstrated slight thickening. Dispersed blood vessels were also noted in the connective tissue (**Fig.12- B, C) (Table 1).**

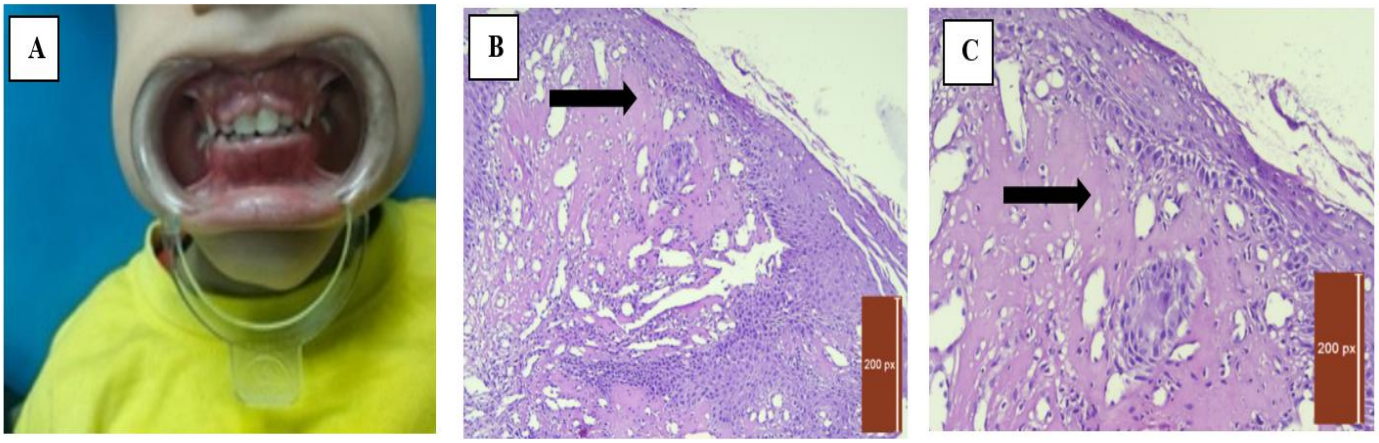


Figure12 JHF A: hyperplastic gingival tissue covering lower teeth. **B, C:** H&E sections showing juxta epithelial hyalinization **(black arrows).**

Little **AB** reaction was noted in scattered fashion (**Fig.13- A).** Intense **PAS** reactivity was observed in areas of hyalinization highlighting the thickened basement membrane (**Fig.13- B, C). MT** and **VG** stains were seen staining the collagen fibers in the connective tissue (**Fig.13- D, E) (Table 2).**

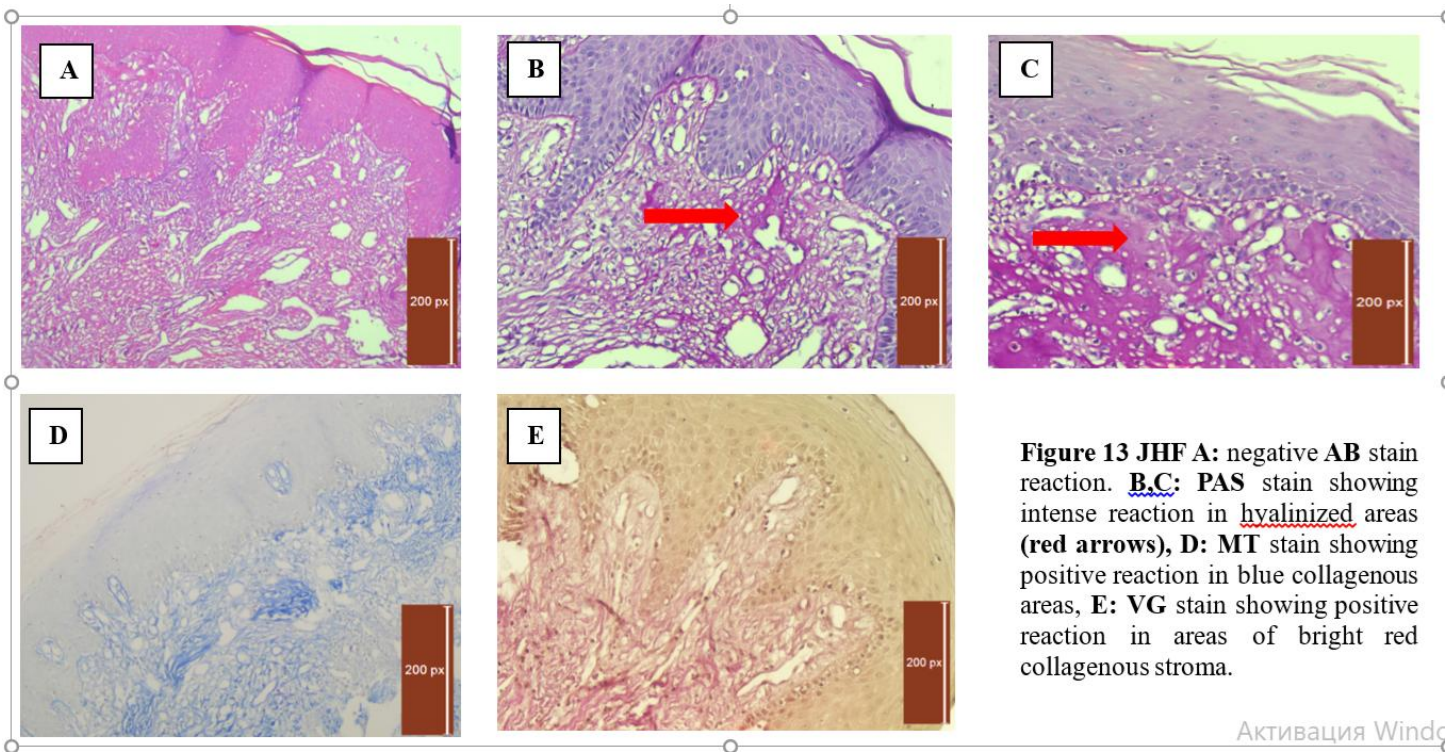


Figure 13 JHF A: negative **AB** stain reaction. **B,C: PAS** stain showing intense reaction in hyalinized areas **(red arrows), D: MT** stain showing positive reaction in blue collagenous areas, **E: VG** stain showing positive reaction in areas of bright red collagenous stroma.

6. **MLs H&E and histochemical stains results:**

Mucolipidosis (MLs) (Fig.14-A) tissue biopsy showed normal thin stratified squamous epithelium. The connective tissue revealed dense collagen fiber bundles and little vascularity. There was no evidence of inflammation **(Fig.14-B) (Table 1).**

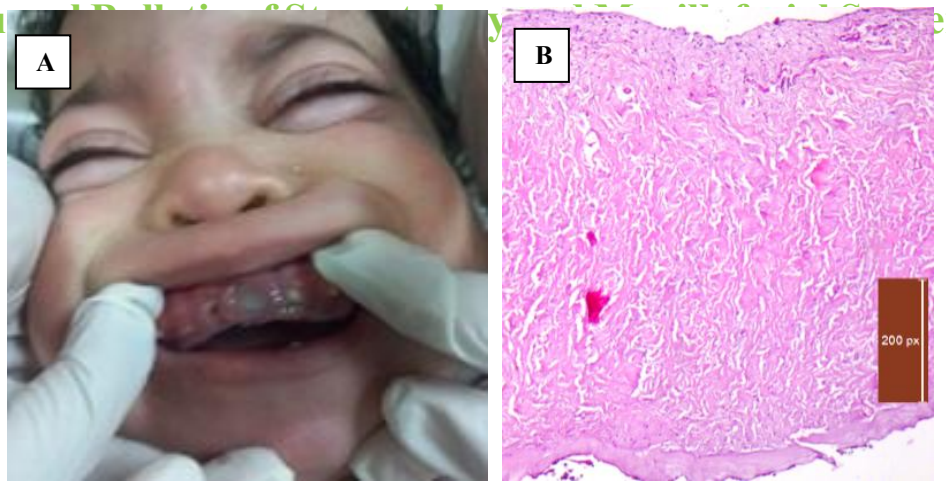


Figure14. MLs A: swollen enlarged gingiva. **B:** H&E section showing heavily collagenous stroma,

AB and **PAS** showed moderate reaction in the connective tissue stroma **(Fig.15-A**), with the latter being weaker **(Fig.15-B).** Identical to **MT, VG** showed heavy reactivity with the dense collagenous stroma **(Fig.15- C,D) (Table 2).**

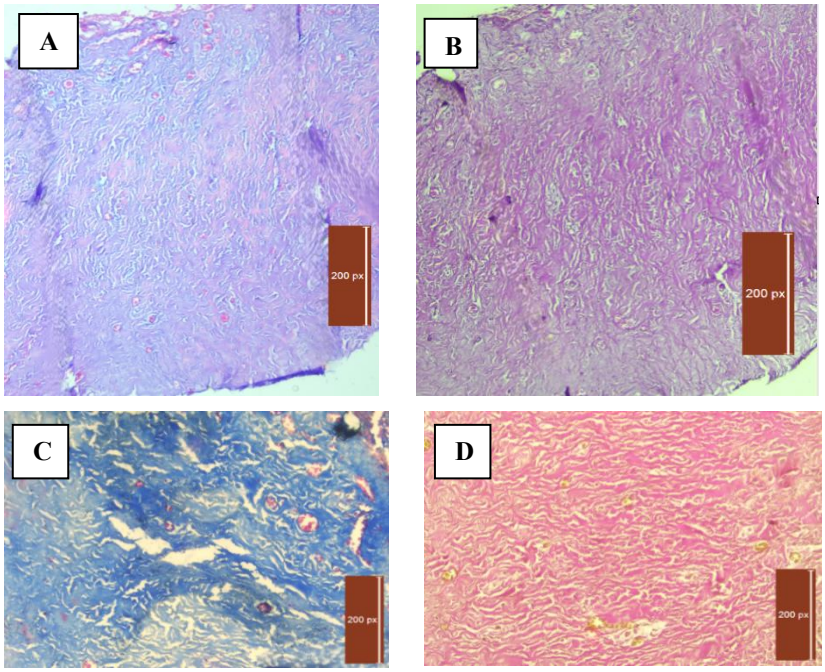


Figure 15. MLs A: AB stain showing scattered reaction in blue, **B: PAS** stain showing weak reaction. **C: MT** stain showing strong reaction with the collagen stromal content taking blue color. **D: VG** stain showing strong reaction with the collagenous content taking bright red color.

Table 1. Showed histopathological findings in H&E sections

Syndromes / Histopathological features		PLS	ERS	JS	WS	JHF	MLs
Epithelial features	Epithelial Hyperplasia	++	++	++	++	+	
	Epithelial Dysplasia	+ **(1 out of 5)**	-	-	-	-	-
	Epithelial Destruction	+ **(1 out of 5)**	+	-	++	-	
	Vacuolization	-	++	-	-	-	-
Thickened Basement Membrane		-	-	-	-	++	-
Sub-epithelial features	Inflammatory cell infiltrate	++ **(4 out of 5)**	+	+	++	-	-
	Hyalinization	-	-	-	-	++	-
	Collagen content (fibromatosis)	+ **(1 out of 5)**	-	+	-	+	++
	Calcifications	-	+	-	-	-	-

Key: **PLS:** Papillon Lefevre Syndrome, **ERS:** Enamel Renal Syndrome, **JS:** Jalili Syndrome, **WS:** Winchester Syndrome, **JHF:** Juvenile Hyaline Fibromatosis, **MLS:** Mucolipidosis,

- : Negative

+ : Positive

++ : Highly Expressed

Table 2. Showed Histochemical findings in special stains sections

Syndromes / Histochemical Stain	Features to be shown by each special stain	PLS	ERS	JS	WS	JHF	MLS
Alcian blue (Blue)	• Acidic mucins • Acid mucopolysaccharides	+	-	-	-	+ (scattered)	+
PAS (Magenta)	• Glycogen • Mucin • Collagen • Proteoglycans in Basement Membrane	+	+	+ BM: ++	+	++	+
Masson Trichrome	• Collagen: Blue/Green • Cytoplasm and Muscles: Red • Nuclei: Black	++	+	++	+	+	++
van- Gieson	• Collagen: Red • Cytoplasm: Yellow • Nuclei: Black/ Blue Black	++	+	++	+	+	++

Key: **PLS:** Papillon Lefevre Syndrome, **ERS:** Enamel Renal Syndrome, **JS:** Jalili Syndrome, **WS:** Winchester Syndrome, **JHF:** Juvenile Hyaline Fibromatosis, **MLS:** Mucolipidosis,
- : Negative
+ : Positive
++ : Intense Staining (Highly Expressed)

Markers levels in GCF results:

When comparing the syndromic group with gingival enlargement to the control group, IL-8 levels showed no statistically significant difference between the two groups ($P = 0.534$) **(Fig. 16)**. The sensitivity and specificity of IL-8 as a diagnostic marker were 72.7% and 56%, respectively, at a cut-off value of 523.2 ng/L **(Table 3).** The overall diagnostic accuracy of IL-8 was calculated to be 61.1% **(Table 3)**.

Table 3. Sensitivity, specificity, predictive values, diagnostic accuracy, Area Under the ROC curve (AUC) and 95% confidence interval (95% CI) of the (AUC) for diagnostic accuracy of IL-8 to differentiate between patients and control groups

Cut-off	Sensitivity %	Specificity %	+PV %	-PV %	Diagnostic accuracy %	AUC		95% CI
≤523.2	72.7	56	42.1	82.4	61.1	0.589		0.413-0.75

In contrast, TNF-α levels were significantly higher in the syndromic group compared to the control group ($P < 0.001$) **(Fig. 16).** At a cut-off value of 598.9 ng/L, TNF-α demonstrated a sensitivity of 72.7% and a specificity of 92% **(Table 4).** Its diagnostic accuracy was calculated as 86.1% **(Table 4)**.

Table 4. Sensitivity, specificity, predictive values, diagnostic accuracy, Area Under the ROC curve (AUC) and 95% confidence interval (95% CI) of the (AUC) for diagnostic accuracy of TNF-α to differentiate between patients and control groups

Cut-off	Sensitivity %	Specificity %	+PV %	-PV %	Diagnostic accuracy %	AUC	95% CI
>598.9	72.7	92	80	88.5	86.1	0.804	0.638-0.917

+PV: Positive Predictive Value, -PV: Negative Predictive Value

MMP-2 levels did not differ significantly between the two groups ($P = 0.369$) **(Fig. 15).** At a cut-off value of 1540.3 ng/mL, MMP-2 had a sensitivity of 63.6% and a specificity of 72% **(Table 5).** The diagnostic accuracy was 69.4% **(Table 5)**.

Table 5. Sensitivity, specificity, predictive values, diagnostic accuracy, Area Under the ROC curve (AUC) and 95% confidence interval (95% CI) of the (AUC) for diagnostic accuracy of MMP-2 to differentiate between patients and control groups

Cut-off	Sensitivity %	Specificity %	+PV %	-PV %	Diagnostic accuracy %	AUC	95% CI
>1540.3	63.6	72	50	81.8	69.4	0.527	0.354-0.695

+PV: Positive Predictive Value, -PV: Negative Predictive Value

For **GAGs,** a statistically significant difference was observed, with the syndromic group showing lower mean levels than the control group ($P < 0.001$) **(Fig. 16).** A cut-off value of ≤48.2 mg/L was identified for distinguishing syndromic cases **(Table 6).** At this threshold, GAGs demonstrated a sensitivity of 100%, a specificity of 92%, and a diagnostic accuracy of 94.4% **(Table 6)**.

Table 6. Sensitivity, specificity, predictive values, diagnostic accuracy, Area Under the ROC curve (AUC) and 95% confidence interval (95% CI) of the (AUC) for diagnostic accuracy of GAGs to differentiate between patients and control groups

Cut-off	Sensitivity %	Specificity %	+PV %	-PV %	Diagnostic accuracy %	AUC	95% CI
≤48.2	100	92	84.6	100	94.4	0.971	0.853-0.999

+PV: Positive Predictive Value, -PV: Negative Predictive Value

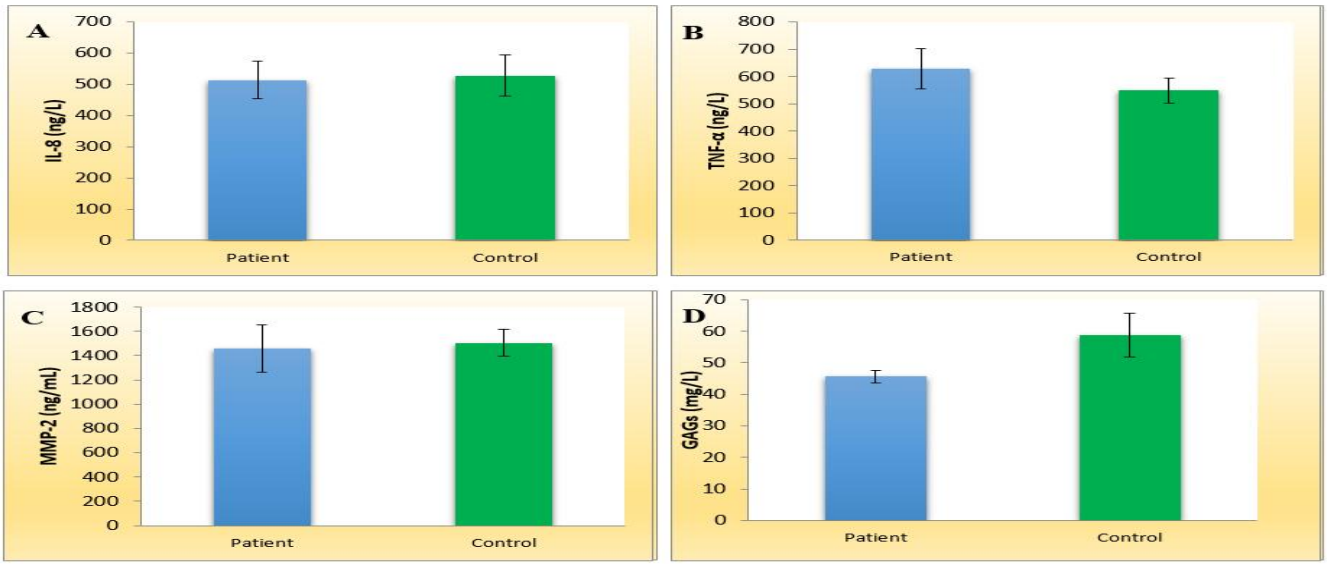


Figure 16 Showing **A:** Comparison between IL-8 levels in patients group and control group. **B:** Comparison between TNF-α levels in patients group and control group. **C:** Comparison between MMP-2 levels in patients

group and control group. **D:** Comparison between GAGs levels in patients group and control group.

ROC curve analysis comparing the diagnostic performance of the four biomarkers revealed that GAGs had the highest diagnostic accuracy (94.4%), followed by TNF-α (86.1%), MMP-2 (69.4%), and IL-8 (61.1%) **(Table 7)**. Pairwise comparisons of the area under the ROC curves (AUCs) showed that GAGs had a significantly higher AUC than both IL-8 and MMP-2 ($P < 0.001$ for both) **(Table 8)**. However, the difference between GAGs and TNF-α was not statistically significant ($P = 0.101$) **(Table 8).**

Table 7. Sensitivity, specificity, predictive values, diagnostic accuracy, Area Under the ROC curve (AUC) and 95% confidence interval (95% CI) of the (AUC) for different markers

Marker	Sensitivity %	Specificity %	+PV %	-PV %	Diagnostic accuracy %	AUC	95% CI
IL-8	72.7	56	42.1	82.4	61.1	0.589	0.413-0.75
MMP-2	63.6	72	50	81.8	69.4	0.527	0.354-0.695
TNF-α	72.7	92	80	88.5	86.1	0.804	0.638-0.917
GAGs	100	92	84.6	100	94.4	0.971	0.853-0.999

Pairwise comparisons of the area under the ROC curves (AUCs) showed that GAGs had a significantly higher AUC than both IL-8 and MMP-2 ($P < 0.001$ for both) **(Table 8)**. However, the difference between GAGs and TNF-α was not statistically significant ($P = 0.101$) **(Table 8).**

Table 8. Results of *z*-test for pair-wise comparisons between Areas under the ROC curve (AUCs) of different markers for differentiation between patients and control

Markers	*z*-statistic	*P*-value
IL-8 vs. MMP-2	0.343	0.731
IL-8 vs. TNF-α	1.255	0.21
IL-8 vs. GAGs	3.632	<0.001*
MMP-2 vs. TNF-α	2.375	0.018*
MMP-2 vs. GAGs	3.502	<0.001*
TNF-α vs. GAGs	1.639	0.101

**: Significant at $P \leq 0.05$*

DISCUSSION

Syndromes with gingival hyperplasia are rare in occurrence and have different prevalence rates. For example, the prevalence of PLS in the general population was reported as 1-4 cases per million. As well as ERS, where its prevalence was reported to be less than 1 case per million [35].

Until 2019, only a total of 134 JS cases and 300 cases of PLS had been reported in the literature [10, 36]. WS is also considered very rare, with only few cases documented [13]. Similarly, the exact prevalence of JHF has not been specified [37]. Due to the wide clinical heterogeneity of MLs, its prevalence was also difficult to determine [38]. These previously reported prevalence data are consistent with our findings, which further confirm the rarity of the syndromes under study.

Over the 18-months duration of our study that conducted in Egypt, PLS was the most frequently encountered syndrome, with a total of 5 cases representing the highest prevalence among the studied conditions. Both ERS and JS cases were diagnosed in 2

cases each, while the remaining syndromes were represented by a single case each.

In the present study, epithelial hyperplasia was observed in almost all tissue specimens. Areas of epithelial destruction were also noted in WS, one case of PLS and ERS. Both changes in the epithelium represent a known reaction for the exaggerated inflammation related to the pathogenesis of these syndromes [39]. In one case of PLS, epithelial hyperplasia was accompanied by mild dysplastic features in the basilar epithelium. This finding was supported by several studies, where in 2018, the incidence of palatal oral squamous cell carcinoma was reported in a PLS patient [40]. Also, ocular surface squamous neoplasia was reported in a case with palmo-plantar keratosis suggestive for PLS [41]. The same patient developed the same lesion in the other eye after 12 years of follow-up and it was also diagnosed as ocular squamous cell carcinoma [42].

Our findings also came in line with those of Al-Benna etal. (2009) [43], who reported the development of squamous cell carcinoma arising from a previous keratotic lesion in a patient with PLS. Additionally, several studies have documented the occurrence of melanoma in PLS patients, developing within their keratotic lesions [44, 45, 46]. However, in a study conducted in 2019, Alsaif, etal. [47] reported a case of basal cell carcinoma developing in non-keratotic skin lesions in a PLS patient.

In the studied case of ERS, epithelial vacuolization was observed. This process can be triggered by various factors, including bacterial toxins, viral infections and certain drugs [48, 49]. Notably, cytoplasmic vacuolization may also occur as a result of wrong tissue fixation [48]. In addition, chronic inflammation has been shown to induce cellular vacuolization, particularly in the basal layer of gingival tissue [50].

In the study of PLS cases, 4 out of 5 patients exhibited intense lymphocytic inflammatory cell infiltration in their gingival biopsies. This finding is consistent with the unique pathophysiology of PLS. Where, the defective neutrophilic function is a central role in disease manifestation [51]. The minimal inflammatory cell infiltrate observed in one PLS case is most likely attributable to the edentulous state of the patient, which may have led to a reduction in inflammatory stimuli and subsequent subsidence of the inflammatory response.

Besides PLS, the WS case also demonstrated an intense inflammatory reaction accompanied by partial destruction of surrounding tissues. The observed gingival enlargement and poor oral hygiene are most probably the underlying cause of this pronounced inflammation [52]. Similarly, the inflammatory responses noted in the remaining syndromic cases are mostly due to inadequate oral hygiene and overall poor oral health status [53].

In the present study, the connective tissue hyalinization was predominantly noted in the JHF case, reflecting the underlying pathogenic mechanism of the disease [54]. Similarly, the calcific bodies identified in ERS cases came in accordance with the frequent calcifications typically seen in both pulpal and gingival tissues of ERS patients [55].

However, the increased collagen production seen in the gingival tissue of the MLs differed from the typical presentation of MLs, which is usually characterized by deposition of GAGs [56]. It is worth noting that, however, that increased collagen deposition in MLs may occur can show in context of chronic inflammation [57].

With regard to histochemical analysis in the current study, AB expression in PLS cases was minimal. This finding reflects the typical structure of gingival tissue in PLS, as AB primarily stains acidic mucins, which is not usually present in this condition [58]. However, Sahni, etal. [59] reported increased AB expression in oral lesions exhibiting epithelial dysplasia. Interestingly, the same study also documented a subsequent decrease in AB expression with the progression to severe dysplasia.

The MLs case examined in this study showed, some expressivity for AB. This came in agreement with Frazier et al. (2008) [60], who illustrated AB staining of sulfated GAGs in MLs cases. On the other hand, the reaction of AB in JHF case came contradicting to the reported nature of the deposited hyaline material, mostly of MMPs and proteoglycans [61]. The negative AB staining observed in the remaining syndromic cases reflects the absence of acidic mucins, aligning with the typical histological structure of their gingival tissues [61].

In the present syndromic cases, PAS reaction was predominantly expressed at the BM area of the epithelium, which is known to be rich in proteoglycans that typically show strong PAS positivity [62]. The thickened basement membrane observed in JS cases may be attributed by the chronic gingivitis which is a

known stimulus for BM multiplication [50].

Conversely, the increased PAS reaction in the JHF case was anticipated, given the proteoglycans content of the hyalinized material characteristic of the condition [61]. In contrast, the remaining syndromic cases exhibited only mild PAS positivity in the stromal tissue, likely reflecting the presence of fine reticular fibers within the stroma [63].

Both MT and VG stains are commonly used to differentiate collagen fibers from other connective tissue components [64]. MT is primarily employed to distinguish muscle fibers (staining red) from collagen fibers (staining blue) [65]. Whereas, VG stain differentiates collagen (staining red) from other tissue elements, which appear yellow [66].

The PLS case with teeth clearance demonstrated high MT expression. This subsequent replacement of inflammation by collagen fibers following teeth removal a valid reason for this intense staining compared to other PLS cases [67]. VG staining showed a similar pattern of increased expression in PLS cases, particularly in the edentulous patient, for the same reason.

Although hyalinization in JHF has been attributed to matrix metalloproteinases (MMPs) and proteoglycans, fibromatosis is recognized as a key histopathological feature of JHF. The strong positivity of both MT and VG stains in the JHF case [68]. Similarly, the MLs case showed

Although hyalinization seen in JHF was thought to be due to MMPs and proteoglycans, fibromatosis proved to be a key feature of JHF. The strong positivity of both MT and VG stains underscores the high collagen content present [68]. Similarly, the MLs case showed marked reactivity with both stains, corresponding to increased collagen deposition, likely secondary to chronic inflammation.

Both Il-8 and TNF-α are well-established pro-inflammatory cytokines involved in the pathogenesis of periodontitis [69]. Previous studies have assessed their levels in PLS patients to investigate their roles in the chemotaxis and activation of polymorphonuclear leukocytes, revealing no statistically significant differences between patient and control groups [70]. These findings are partially aligned with our results, where IL-8 levels in GCF showed no significant difference between patient and control groups.

In contrast, TNF-α levels in our study demonstrated a statistically significant increase towards the patient group compared to controls. This elevation is likely due to the heightened inflammatory response observed in the majority of cases. Contributing factors such as gingival enlargement and poor oral hygiene appear to play a central role in driving gingival inflammation and, consequently, elevated TNF-α levels [71].

The ECM proteolytic enzymes known to promote ECM modulation and enhance angiogenesis are MMP-2 [72]. Although being a core driver enzyme in the inflammatory process and its mutation is directly involved in the pathogenesis of WS and JHF [73, 74], MMP-2 did not show statistically significant differences between patient and control groups in the present study. This lack of significance may be attributed to the heterogeneity of the syndromes studied, each characterized by distinct pathophysiological mechanisms that likely influence MMP-2 levels differently.

Among ECM polysaccharides responsible for the cellular interactions with the surrounding microenvironment are GAGs [75]. They are involved in the process of cellular adhesion, migration and differentiation. Moreover, their contribution to homeostasis and angiogenesis has also been confirmed by Wang and Chi (2022) [76]. At the sites of increased mechanical stresses, ECM automatically replaces proteoglycans by GAGs to enhance resistance under physiological conditions [77].

Surprisingly, in the current study, GAGs levels in the GCF were lower in syndromic patients compared to healthy controls. This decrease in GAGs levels may be explained as a disruption in their ECM due to the underlying pathology in each syndrome [78, 79]. Additionally, the increased collagen production observed in the MLS case could contribute to altered ECM composition, subsequently affecting GAGs levels.

Notably, GAGs demonstrated the highest diagnostic accuracy among all studied biomarkers, however, the expression was higher in the control group rather than the syndromic group. While GAGs are already established diagnostic markers in Mucopolysaccharidosis and MLs cases [80], to our knowledge, this is the first study to assess their diagnostic performance across a broader range of rare

syndromes.

The marker that ranked second in diagnostic accuracy was TNF-α, with elevated expression in the syndromic group. As a pro-inflammatory cytokine, its presence aligns with the observed inflammatory profiles in most patients. However, previous studies have shown that TNF-α levels in the GCF of PLS patients do not significantly differ between active and non-active disease states [81]. Similarly, while cytokine profiles have been explored in MLs patients, TNF-α was not among the cytokines associated with motor dysfunction in these cases [82].

CONCLUSIONS

Hematoxylin and Eosin (H&E) staining could be considered a valuable tool for the preliminary histopathological diagnosis of PLS, ERS, and JHF, owing to the distinctive microscopic features observed in each. In PLS, the presence of lymphoplasmacytic infiltrates, when considered alongside hallmark clinical findings such as palmo-plantar keratosis and early-onset periodontitis, supports accurate diagnosis. Additional dysplastic changes observed in the epithelium can further ensure the suspicion.

In ERS, the combination of pulpal calcifications, nephrocalcinosis, and AI, together with gingival calcifications evident on histopathology, enhances diagnostic confidence. Similarly, JHF is characterized by prominent connective tissue hyalinization, which, in conjunction with cutaneous nodular lesions, aids in distinguishing this condition.

In contrast, the histopathological features observed JS, WS, and MLS were non-specific and therefore of limited diagnostic utility.

Histochemical techniques applied to the syndromic cases studied did not significantly contribute to diagnostic clarity. Variability in molecular expression according to disease stage undermined their reliability. For example, while PAS positivity was elevated in JHF cases, it did not provide additional information beyond what was observed with routine H&E staining. Likewise, collagen assessment using MT and VG stains yielded non-specific results across all syndromes.

ELISA analysis of GCF demonstrated that TNF-α had the highest sensitivity, specificity, and diagnostic accuracy among the tested markers (TNF-α, IL-8, and MMP-2), highlighting its potential as a supportive diagnostic biomarker. Although GAGs showed consistently elevated levels across multiple parameters, their paradoxically higher levels in control samples compared to patient groups limit their use in diagnosis and suggest a possible role in disease exclusion rather than confirmation.

Recommendations

Further studies are warranted to elucidate the dysplastic changes observed in PLS, with the goal of reducing the future risk of malignant transformation. This could be achieved through the implementation of regular screening programs and long-term follow-up protocols.

Additionally, future research should include a larger number of cases for each syndrome to ensure sufficient statistical power and to validate the diagnostic utility of each marker across different syndromes.

Moreover, further investigations are needed to confirm the role of TNF-α in each syndrome individually. The potential of TNF-α as a prognostic marker also merits comprehensive evaluation in these cases.

DECLARATION

Conflicts of interest and financial disclosures
The author declares that he has no conflict percent and there was no external source of funding for the research in question.
Ethical approval
This work was approved by the Research Ethical Committee at the Faculty of Dentistry, Cairo University, done in compliance with the Helsinki Declaration, and written informed consent was obtained from the patient.
Informed consent
Informed consent was obtained from all individual participants included in the study.
Source of funding
The work was not funded.

REFERENCES

[1] Salerno C, D'Avola V, Oberti L, Almonte E, Bazzini EM, Tartaglia GM, Cagetti MG. Rare Genetic Syndromes and Oral Anomalies: A Review of the Literature and Case Series with a New Classification Proposal. Children (Basel). 2021 Dec 26;9(1):12. doi: 10.3390/children9010012. PMID: 35053637; PMCID: PMC8774676.

[2] De Falco, D., Della Vella, F., Scivetti, M., Suriano,

C., De Benedittis, M., & Petruzzi, M. (2022). Non-Plaque Induced Diffuse Gingival Overgrowth: An Overview. *Applied Sciences*, *12*(8), 3731. https://doi.org/10.3390/app12083731 .

[3] Agrawal, A. A. (2015). "Gingival enlargements: Differential diagnosis and review of literature." World J Clin Cases **3**(9): 779-788.

[4] Costa, C. R. R., S. V. Braz, I. P. de Toledo, H. Martelli-Júnior, J. F. Mazzeu, E. N. S. Guerra, R. D. Coletta and A. C. Acevedo (2021). "Syndromes with gingival fibromatosis: A systematic review." Oral Diseases **27**(4): 881-893.

[5] Phull, T., Jyoti, D., Malhotra, R., Nayak, S., Modi, H., Singla, I., & P, A. (2023). Diagnosis and Management of Papillon-Lefevre Syndrome: A Rare Case Report and a Brief Review of Literature. *Cureus*, *15*(8), e43335. https://doi.org/10.7759/cureus.43335.

[6] Raja Rajeswari, K., Almansour, R., Alrajhi, F., Fahad Binmeqren, A., Shayan Albaqami, M., & Abdullah Albarrak, R. (2024). Papillon-Lefèvre syndrome in dental pediatric patient: A comprehensive review. *The Saudi dental journal*, *36*(5), 682–687. https://doi.org/10.1016/j.sdentj.2024.02.003.

[7] Machado, R. A., Cuadra-Zelaya, F. J. M., Martelli-Júnior, H., Miranda, R. T., Casarin, R. C. V., Corrêa, M. G., Nociti, F., & Coletta, R. D. (2019). Clinical and molecular analysis in Papillon-Lefèvre syndrome. *American journal of medical genetics. Part A*, *179*(10), 2124–2131. https://doi.org/10.1002/ajmg.a.61285.

8] Dalgıc, B., Bukulmez, A., & Sarı, S. (2011). Eponym: Papillon–Lefevre syndrome. *European journal of pediatrics*, *170*(6), 689-691.

[9] Erkapers, M., Frykholm, C., Furuland, H., Segerström, S., & Thor, A. (2024). A case of enamel renal syndrome from a novel genetic mutation, multidisciplinary management and long-term prognosis. *Upsala journal of medical sciences*, *129*, 10.48101/ujms.v129.10228. https://doi.org/10.48101/ujms.v129.10228.

[10] Daneshmandpour, Y., H. Darvish, F. Pashazadeh and B. Emamalizadeh (2019). "Features, genetics and their correlation in Jalili syndrome: a systematic review." J Med Genet **56**(6): 358-369.

[11] Jalili I. K. (2010). Cone-rod dystrophy and amelogenesis imperfecta (Jalili syndrome): phenotypes and environs. *Eye (London, England)*, *24*(11), 1659–1668. https://doi.org/10.1038/eye.2010.103.

[12] Evans, B. R., Mosig, R. A., Lobl, M., Martignetti, C. R., Camacho, C., Grum-Tokars, V., ... & Martignetti, J. A. (2012). Mutation of membrane type-1 metalloproteinase, MT1-MMP, causes the multicentric osteolysis and arthritis disease Winchester syndrome. The American Journal of Human Genetics, 91(3), 572-576.

[13] Chen, H. (2017). Winchester Syndrome. Atlas of Genetic Diagnosis and Counseling. H. Chen. New York, NY, Springer New York**:** 2995-3000.

[14] Xia, L., Hu, Y., Zhang, C., Wu, D., & Chen, Y. (2021). Juvenile hyaline fibromatosis: a rare oral disease case report and literature review. *Translational pediatrics*, *10*(11), 3124–3129. https://doi.org/10.21037/tp-21-169.

[15] Knežević, P., Tarle, M., Fratrić, L. I., Tarle, A., Knežević-Krajina, H., & Macan, D. (2020). Gingival Hypertrophy in a Child with Hyaline Fibromatosis Syndrome. *Acta stomatologica Croatica*, *54*(1), 69–74. https://doi.org/10.15644/asc54/1/8.

[16] de Bode, C. J., E. J. Dogterom, A. V. J. Rozeboom, J. J. Langendonk, E. B. Wolvius, A. T. van der Ploeg, E. Oussoren and M. Wagenmakers (2022). "Orofacial abnormalities in mucopolysaccharidosis and mucolipidosis type II and III: A systematic review." JIMD Rep **63**(6): 621-629.

[17] Monteagudo-Vilavedra, E., Rodrigues, D., Vella, G., Bravo, S. B., Pena, C., Lopez-Valverde, L., Colon, C., Sanchez-Pintos, P., Otero Espinar, F. J., Couce, M. L., & Alvarez, J. V. (2025). Novel Phenotypical and Biochemical Findings in Mucolipidosis Type II. *International journal of molecular sciences*, *26*(6), 2408. https://doi.org/10.3390/ijms26062408.

[18] Gawron, K., K. Łazarz-Bartyzel, J. Potempa and M. Chomyszyn-Gajewska (2016). "Gingival fibromatosis: clinical, molecular and therapeutic issues." Orphanet Journal of Rare Diseases **11**(1): 9.

[19] Goswami, R. S. and S. Harada (2020). "An Overview of Molecular Genetic Diagnosis Techniques." Curr Protoc Hum Genet **105**(1): e97.

[20] Riva, M. A., M. Manzoni, G. Isimbaldi, G. Cesana and F. Pagni (2014). "Histochemistry: historical development and current use in pathology." Biotech Histochem **89**(2): 81-90.

[21] Calvi, E. N. d. C., F. X. Nahas, M. V. Barbosa, J. A. Calil, S. S. M. Ihara, M. d. S. Silva, M. F. d. Franco and L. M. Ferreira (2012). "An experimental model for the study of collagen fibers in skeletal muscle." Acta cirurgica brasileira **27**: 681-686.

[22] Mudasir, Z., S. Adil and A. I. Edriss (2016). "Comparison between Giemsa and Van Geison stains in demonstration of collagen fibers (Kosti-2016)." African Journal of Biotechnology **15**(37): 2063-2067.

[23] Fu, D. A. and M. Campbell-Thompson (2017). "Periodic acid-Schiff staining with diastase." Alpha-1 Antitrypsin Deficiency: Methods and Protocols: 145-149.

[24] Dong, W., Y.-k. Matsuno and A. Kameyama (2012). "A procedure for Alcian blue staining of mucins on polyvinylidene difluoride membranes." Analytical chemistry **84**(20): 8461-8466.

[25] Nazar Majeed, Z., K. Philip, A. M. Alabsi, S. Pushparajan and D. Swaminathan (2016). "Identification of Gingival Crevicular Fluid Sampling, Analytical Methods, and Oral Biomarkers for the Diagnosis and Monitoring of Periodontal Diseases: A Systematic Review." Dis Markers **2016**: 1804727.

[26] Fatima, T., Z. Khurshid, A. Rehman, E. Imran, K. C. Srivastava and D. Shrivastava (2021). "Gingival Crevicular Fluid (GCF): A Diagnostic Tool for the Detection of Periodontal Health and Diseases." Molecules **26**(5).

[27] Lee, H. S. and W. J. Kim (2022). "The Role of Matrix Metalloproteinase in Inflammation with a Focus on Infectious Diseases." Int J Mol Sci **23**(18).

[28] Arunkumar, N., D. C. Vu, S. Khan, H. Kobayashi, T. B. Ngoc Can, T. Oguni, J. Watanabe, M. Tanaka, S. Yamaguchi, T. Taketani, Y. Ago, H. Ohnishi, S. Saikia, J. V. Álvarez and S. Tomatsu (2021) "Diagnosis of Mucopolysaccharidoses and Mucolipidosis by Assaying Multiplex Enzymes and Glycosaminoglycans." Diagnostics **11** DOI: 10.3390/diagnostics11081347.

[29] Leal, A. F., E. Benincore-Flórez, E. Rintz, A. M. Herreño-Pachón, B. Celik, Y. Ago, C. J. Alméciga-Díaz and S. Tomatsu (2023) "Mucopolysaccharidoses: Cellular Consequences of Glycosaminoglycans Accumulation and Potential Targets." International Journal of Molecular Sciences **24** DOI: 10.3390/ijms24010477.

[30] Hornbeck P. V. (2015). Enzyme-Linked Immunosorbent Assays. *Current protocols in immunology*, *110*, 2.1.1–2.1.23. https://doi.org/10.1002/0471142735.im0201s110.

[31] Feldman, A. T., & Wolfe, D. (2014). Tissue processing and hematoxylin and eosin staining. *Methods in molecular biology (Clifton, N.J.)*, *1180*, 31–43. https://doi.org/10.1007/978-1-4939-1050-2_3.

[32] Arunachalam, K., Sreeja, P.S. (2025). Alcian Blue Staining Protocol. In: Advanced Cell and Molecular Techniques. Springer Protocols Handbooks. Humana, New York, NY.

[33] Denn, A., & Mikulski, Z. (2022). Masson's Trichrome Staining Protocol for FFPE spheroids and microtissues. https://doi.org/10.1007/978-1-0716-4518-5_38.\

[34] Dey, P. (2018). Connective Tissue Stain: Principle and Procedure. In: Basic and Advanced Laboratory Techniques in Histopathology and Cytology. Springer, Singapore. https://doi.org/10.1007/978-981-10-8252-8_10.

[35] Roomaney, I. A., S. Kabbashi and M. Chetty (2025). "The craniofacial, dental and systemic manifestations of Enamel Renal Syndrome: A Scoping review." European Journal of Medical Genetics **75**: 105008.

[36] Hattab FN. Papillon-Lefèvre syndrome: from then until now. *Stomatological Dis Sci*. 2019;3:1. http://dx.doi.org/10.20517/2573-0002.2018.22.

[37] Syamala, S. R., P. Sreenivasan and S. V. Hariharan (2021). Hyaline Fibromatosis Syndrome. Indian J Pediatr. India. **88:** 614.

[38] Cavalcante, W. C., L. C. Santos, J. N. Dos Santos, S. J. de Vasconcellos and R. A. de Azevedo (2012).

"Oral findings in patients with mucolipidosis type III." Braz Dent J **23**(4): 461-466.

[39] Hunasgi, S., A. Koneru, M. Vanishree and V. Manvikar (2017). "Assessment of reactive gingival lesions of oral cavity: A histopathological study." Journal of Oral and Maxillofacial Pathology **21**(1).

[40] Aziz, M., M. Shawky and M. Atef (2018). "Oral Squamous Cell Carcinoma Associated with Papillon-Lefevre Syndrome: Systematic Review and the First Reported Case." **4**: 31-35.

[41] Murthy, R., S. G. Honavar, G. K. Vemuganti, S. Burman, M. Naik and A. Parathasaradhi (2005). "Ocular surface squamous neoplasia in Papillon–Lefevre syndrome." American Journal of Ophthalmology **139**(1): 207-209.

[42] Kaliki, S., S. Singh, S. Gowrishankar and V. A. P. Reddy (2017). "Ocular Surface Squamous Neoplasia in Papillon-Lefèvre Syndrome: Outcome at Long-Term Follow-Up of 12 Years." Cornea **36**(6): 743-746.

[43] Al-Benna, S., R. Hasler, I. Stricker, H. U. Steinau and L. Steinstraesser (2009). "Papillon-Lefèvre syndrome and squamous cell carcinoma: a case report." Cases J **2**: 7067.

[44] Hacham-Zadeh, S. and L. Goldberg (1982). "Malignant melanoma and Papillon-Lefvre syndrome." Arch Dermatol **118**(1): 2.

[45] Nakajima, K., H. Nakano, N. Takiyoshi, A. Rokunohe, S. Ikenaga, T. Aizu, T. Kaneko, Y. Mitsuhashi and D. Sawamura (2008). "Papillon-Lefèvre syndrome and malignant melanoma. A high incidence of melanoma development in Japanese palmoplantar keratoderma patients." Dermatology **217**(1): 58-62.

[46] Cook, G. P. (2009). Papillon-Lefèvre syndrome and malignant melanoma. Dermatology. Switzerland. **219:** 187-188.

[47] Alsaif, F. M., M. A. Arafah, R. T. Alenazi and G. F. Alotaibi (2019). "Papillon-Lefèvre Syndrome and Basal Cell Carcinoma: A Case Study." Case Rep Oncol **12**(2): 411-417.

[48] Henics, T., & Wheatley, D. N. (1999). Cytoplasmic vacuolation, adaptation and cell death: a view on new perspectives and features. *Biology of the cell*, *91*(7), 485–498. https://doi.org/10.1016/s0248-4900 (00)88205-2.

[49] Shubin, A. V., Demidyuk, I. V., Komissarov, A. A., Rafieva, L. M., & Kostrov, S. V. (2016). Cytoplasmic vacuolization in cell death and survival. *Oncotarget*, *7*(34), 55863–55889. https://doi.org/10.18632/oncotarget.10150

[50] Lushnikova, E. L., Nepomnyashchikh, L. M., Oskolsky, G. I., & Jurkevich, N. V. (2012). Ultrastructure of gingival epithelium in chronic gingivitis. *Bulletin of experimental biology and medicine*, *152*(5), 637–641. https://doi.org/10.1007/s10517-012-1596-9.

[51] Korkmaz, B., Caughey, G. H., Chapple, I., Gauthier, F., Hirschfeld, J., Jenne, D. E., Kettritz, R., Lalmanach, G., Lamort, A. S., Lauritzen, C., Łęgowska, M., Lesner, A., Marchand-Adam, S., McKaig, S. J., Moss, C., Pedersen, J., Roberts, H., Schreiber, A., Seren, S., & Thakker, N. S. (2018). Therapeutic targeting of cathepsin C: from pathophysiology to treatment. *Pharmacology & therapeutics*, *190*, 202–236. https://doi.org/10.1016/j.pharmthera.2018.05.011

[52] Sollazzo, V., & Sollazzo, V. (2023). WINCHESTER SYNDROME: A SHORT REVIEW. *Eur J Musculoskel Dis*, *12*(1), 33-37.

[53] Piekoszewska-Ziętek, P., Witt-Porczyk, A., Turska-Szybka, A., & Olczak-Kowalczyk, D. (2024). Hygienic behaviors and use of dental care in patients with genetic syndromes. *Scientific reports*, *14*(1), 30756. https://doi.org/10.1038/s41598-024-80922-0

[54] Momin, Y. A., B. M. Bharambe and G. D'Costa (2011). "Juvenile hyaline fibromatosis: A rare lesion." Indian Journal of Pathology and Microbiology **54**(4).

[55] Khalifa, R., R. Kammoun, L. Mansour, T. Ben Alaya and S. Ghoul (2024). "Enamel renal syndrome: A case report with calcifications in pulp, gingivae, dental follicle and kidneys." Spec Care Dentist **44**(3): 722-728.

[56] Khan, S. A. and S. C. Tomatsu (2020). "Mucolipidoses Overview: Past, Present, and Future." Int J Mol Sci **21**(18).

[57] Almeida, T., T. Valverde, P. Martins-Junior, H. Ribeiro, G. Kitten and L. Carvalhaes (2015). "Morphological and quantitative study of collagen fibers in healthy and diseased human gingival tissues." Rom J Morphol Embryol **56**(1): 33-40.

[58] Vadivazhagan, K., K. Amitkumar, M. Sudalaimuthu and K. Vani (2022). "Quantitative analysis of mucin expression using combined alcian blue-periodic acid schiff (AB-PAS) stain and combined high iron diamine-alcian blue (HID-AB) stain and the correlation with histomorphological score in chronic calculous cholecystitis." Cureus **14**(11).

[59] Sahni, A., Rehani, S., Kardam, P., Sethi, S., Kumari, R., & Mathias, Y. (2019). Analysis of stromal mucin in oral epithelial dysplasia & oral squamous cell carcinoma- A histochemical study. *Journal of oral biology and craniofacial research*, *9*(1), 40–46. https://doi.org/10.1016/j.jobcr.2018.07.004

[60] Frazier, S. B., Roodhouse, K. A., Hourcade, D. E., & Zhang, L. (2008). The Quantification of Glycosaminoglycans: A Comparison of HPLC, Carbazole, and Alcian Blue Methods. *Open glycoscience*, *1*, 31–39. https://doi.org/10.2174/1875398100801010031

[61] Marques, S. A., Stolf, H. O., Polizel, J. O., Munhoz, T., Brandão, M. C., & Marques, M. E. (2016). Hyaline fibromatosis syndrome: cutaneous manifestations. *Anais brasileiros de dermatologia*, *91*(2), 226–229. https://doi.org/10.1590/abd1806-4841.20163799

[62] Ponmar, M., Sathishkumar, D., Pulimood, S. A., & Thomas, M. (2024). Patterns of Periodic Acid–Schiff Staining in Subepidermal Bullous Disorders with Emphasis on Bullous Pemphigoid. *Current Medical Issues*, *22*(1), 1-6.

[63] Dey, P. (2018). Basic and advanced laboratory techniques in histopathology and cytology.

[64] Kanneganti, S., Kattappagari, K. K., Tanuja, K., Chandra, K. L. P., Poosarla, C., & Baddam, V. R. (2018). Oral submucous fibrosis: Clinical and histopathological correlation of collagen fibers using Masson's trichrome and Van Gieson stains. *Journal of Dr. YSR University of Health Sciences*, *7*(3), 181-184.

[65] Van De Vlekkert, D., Machado, E., & d'Azzo, A. (2020). Analysis of Generalized Fibrosis in Mouse Tissue Sections with Masson's Trichrome Staining. *Bio-protocol*, *10*(10), e3629. https://doi.org/10.21769/BioProtoc.3629.

[66] Segnani, C., Ippolito, C., Antonioli, L., Pellegrini, C., Blandizzi, C., Dolfi, A., & Bernardini, N. (2015). Histochemical Detection of Collagen Fibers by Sirius Red/Fast Green Is More Sensitive than van Gieson or Sirius Red Alone in Normal and Inflamed Rat Colon. *PloS one*, *10*(12), e0144630. https://doi.org/10.1371/journal.pone.0144630

[67] Patel, V. I., Thakkar, K. R., Gupta, S. S., & Pujara, M. J. (2024). Papillon-Lefevre syndrome: Case series. *Journal of oral and maxillofacial pathology : JOMFP*, *28*(4), 694–700. https://doi.org/10.4103/jomfp.jomfp_25_24

[68] Casas-Alba, D., Martínez-Monseny, A., Pino-Ramírez, R. M., Alsina, L., Castejón, E., Navarro-Vilarrubí, S., Pérez-Dueñas, B., Serrano, M., Palau, F., & García-Alix, A. (2018). Hyaline fibromatosis syndrome: Clinical update and phenotype-genotype correlations. *Human mutation*, *39*(12), 1752–1763. https://doi.org/10.1002/humu.23638

[69] Noh, M. K., Jung, M., Kim, S. H., Lee, S. R., Park, K. H., Kim, D. H., Kim, H. H., & Park, Y. G. (2013). Assessment of IL-6, IL-8 and TNF-α levels in the gingival tissue of patients with periodontitis. *Experimental and therapeutic medicine*, *6*(3), 847–851. https://doi.org/10.3892/etm.2013.1222

[70] Sadik, C. D., Noack, B., Schacher, B., Pfeilschifter, J., Mühl, H., & Eickholz, P. (2012). Cytokine production by leukocytes of Papillon-Lefèvre syndrome patients in whole blood cultures. *Clinical oral investigations*, *16*(2), 591–597. https://doi.org/10.1007/s00784-011-0532-0

[71] Gür, B., Afacan, B., Çevik, Ö., Köse, T., & Emingil, G. (2023). Gingival crevicular fluid periodontal ligament-associated protein-1, sclerostin, and tumor necrosis factor-alpha levels in periodontitis. *Journal of periodontology*, *94*(10), 1166–1175. https://doi.org/10.1002/JPER.22-0750

[72] Checchi, V., Maravic, T., Bellini, P., Generali, L., Consolo, U., Breschi, L., & Mazzoni, A. (2020). The

Role of Matrix Metalloproteinases in Periodontal Disease. *International journal of environmental research and public health*, *17*(14), 4923. https://doi.org/10.3390/ijerph17144923

[73] Nithya Rangasamy, Nachimuthu Senthil Kumar, Santhy K.S., Computational analysis of missense variants in MMP2 gene linked with Winchester syndrome and Nodulosis-Arthropathy-Osteolysis reveals structural shift in protein-protein and protein-ligand complexes, Meta Gene, Volume 29, 2021, 100931, ISSN 2214-5400, https://doi.org/10.1016/j.mgene.2021.100931.

[74] Tzellos, T. G., Batzios, S. P., Dionyssopoulos, A., Karakiulakis, G., & Papakonstantinou, E. (2011). Differential expression of matrix metalloproteinases and proteoglycans in Juvenile Hyaline Fibromatosis. *Journal of dermatological science*, *61*(2), 94–100. https://doi.org/10.1016/j.jdermsci.2010.12.002**.**

[75] Ricard-Blum, S., & Perez, S. (2022). Glycosaminoglycan interaction networks and databases. *Current opinion in structural biology*, *74*, 102355. https://doi.org/10.1016/j.sbi.2022.102355.

[76] Yeh, L. R., Zhang, Y., Chen, J. H., Liu, Y. L., Wang, A. C., Yang, J. Y., Yeh, W. C., Cheng, C. S., Chen, L. K., & Su, M. Y. (2022). A deep learning-based method for the diagnosis of vertebral fractures on spine MRI: retrospective training and validation of ResNet. *European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society*, *31*(8), 2022–2030. https://doi.org/10.1007/s00586-022-07121-1

[77] Melrose, J. (2025) "Glycosaminoglycans, Instructive Biomolecules That Regulate Cellular Activity and Synaptic Neuronal Control of Specific Tissue Functional Properties." International Journal of Molecular Sciences **26** DOI: 10.3390/ijms26062554.

[78] Ennemoser, M., Pum, A., & Kungl, A. (2022). Disease-specific glycosaminoglycan patterns in the extracellular matrix of human lung and brain. *Carbohydrate research*, *511*, 108480. https://doi.org/10.1016/j.carres.2021.108480

[79] Roth, J., Hoop, C. L., Williams, J. K., Hayes, R., & Baum, J. (2023). Probing the effect of glycosaminoglycan depletion on integrin interactions with collagen I fibrils in the native extracellular matrix environment. *Protein science : a publication of the Protein Society*, *32*(1), e4508. https://doi.org/10.1002/pro.4508

[80] Kubaski, F., Suzuki, Y., Orii, K., Giugliani, R., Church, H. J., Mason, R. W., Dũng, V. C., Ngoc, C. T., Yamaguchi, S., Kobayashi, H., Girisha, K. M., Fukao, T., Orii, T., & Tomatsu, S. (2017). Glycosaminoglycan levels in dried blood spots of patients with mucopolysaccharidoses and mucolipidoses. *Molecular genetics and metabolism*, *120*(3), 247–254. https://doi.org/10.1016/j.ymgme.2016.12.010

[81] Ullbro, C., C. G. Crossner, T. Nederfors, R. Parhar, F. Al Mohanna, M. C. Meikle, J. J. Reynolds and S. Twetman (2004). "Cytokines, matrix metalloproteinases and tissue inhibitor of metalloproteinases-1 in gingival crevicular fluid from patients with Papillon-Lefèvre syndrome." Acta Odontol Scand **62**(2): 70-74.

[82] Misko, A. L., L. D. Weinstock, S. B. Sankar, A. Furness, Y. Grishchuk and L. B. Wood (2022). "Peripheral Inflammatory Cytokine Signature Mirrors Motor Deficits in Mucolipidosis IV." Cells **11**(3).

Abbreviations:

AB: Alcian Blue, **AI:** Amelogenesis Imperfecta, **ANTXR2:** Anthrax toxin receptor 2, **AR:** Autosomal Recessive, **AUC:** Area Under the ROC curve, **CRD:** Cone-Rod Dystrophy, **ELISA:** Enzyme Linked Immunosorbent Assay, **ERS:** Enamel Renal Syndrome, **GAGs:** Glycosaminoglycans, **GCF:** Gingival Crevicular Fluid, **H&E:** Hematoxylin and Eosin, **IL-8:** Interlukin-8, **JHF:** Juvenile Hyaline Fibromatosis, **JS:** Jalili Syndrome, **MLs:** Mucolipidosis Syndrome, **MMP-2:** Matrix-metalloproteinase-2, **MT:** Masson Trichrome, **PAS:** Periodic Acid Schiff, **PCR:** Polymerase Chain Reaction, **PLS:** Papillon Lefevre Syndrome, **VG:** Van Gieson, **WS:** Winchester Syndrome, **95% CI:** 95% Confidence Interval.