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التحري الميكروبي باستخدام تقنية Real Time PCR وتقييم السيتوكينات في مرضى التهاب ما حول سن العقل

رسالة مقدمة إلـــى
مجلس كلية طب الأسنان/ جامعة بابل كجزء من متطلبات
نيل درجة الماجستير في الأحياء المجهرية الفموية والطبية

من قبل

احمد نبيل قاسم الحبوبي

بكالوريوس طب وجراحة الفم والاسنان (2009)

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الخلاصة:-

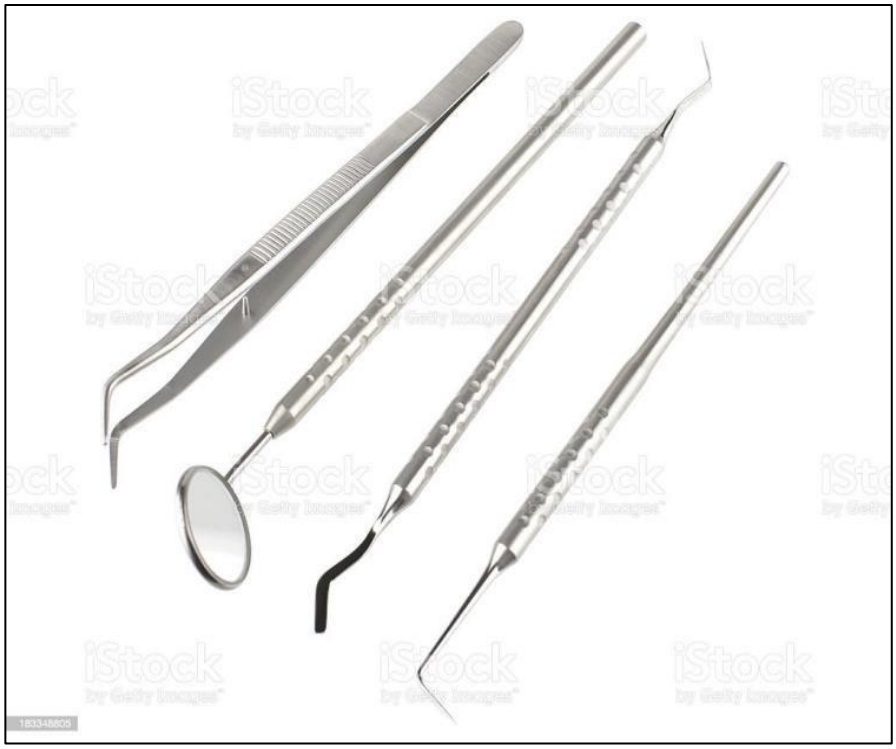
يشير مصطلح "التهاب حوائط التاج" إلى حالة التهاب في الأنسجة الرخوة في الفم، والتي تحدث نتيجة لإصابة اللثة والجيوب اللثوية بالقرب من سن غير ناضج. عادةً ما تتسارع هذه العملية بسبب تراكم بقايا الطعام بالقرب من التاج والإصابات التي تسببها الأسنان المعاكسة في الأنسجة المحيطة به. يعتبر التهاب حوائط التاج التهابًا يؤثرفي الأنسجة الرخوة المحيطة بتاج السن الثالث في الفك السفلي، سواء كان السن جزئيًا ظاهرا او كليا مطمور، أيضا يحدث هذا الالتهاب نتيجة تراكم بكتيريا الفم المتنوعة. تهدف هذه الدراسة إلى تحديد البكتيريا الرئيسية المسببة لالتهاب حوائط التاج وتقييم مستويات الانترلوكين-8 وعامل النمو التحويلي بيتا-1 في منطقة التهاب حوائط التاج . تم الحصول على موافقة جميع المشاركين في هذه الدراسة وفقًا لنموذج الموافقة المعتمد. تم تشخيص خمسين مريضًا (50) بالتهاب حوائط التاج، وهم (33) أنثى و (17) ذكرًا، بينما تم اختيار ثلاثين بالغًا (30) سليمًا يعملون كمجموعة ضابطة في الدراسة. تم تضمين الأفراد البالغين الذين تتجاوز أعمارهم 16 عامًا في هذه الدراسة .

تم جمع عينات من نسيج التهاب حوائط التاج عن طريق عزل الضرس الثالث السفلي وتجفيف المنطقة باستخدام حقنة هوائية، ثم تم إدخال نقطة ورقية في جيوب التاج حتى تم الشعور بالمقاومة لمدة 30 ثانية، ثم تم وضع النقطة الورقية في أنبوب إيبندورف يحتوي على محلول فوسفات وتخزينه في درجة حرارة (-20 درجة مئوية) في الفريزر العميق حتى يتم استخدامها في تقنية تفاعل البلمرة الوقت الحقيقي. تم جمع عينات اللعاب عن طريق غسل الفم بماء مقطر (10 مل) لمدة 30-60 ثانية لضمان إزالة أي رواسب. ثم بعد دقيقتين من تراكم اللعاب في فم المريض تم ارشاد المريض لبصق اللعاب المجمع في الوعاء المستقبل. تم تركيز عينات اللعاب غير المحفزة التي تحتوي على 2 مل في جهاز الطرد المركزي لمدة 15 دقيقة بسرعة 4000 دورة في الدقيقة؛ تم سحب السوائل الفوقية الشفافة عن طريق شفطها ووضعها في أنابيب عادية لكي تُحفظ في درجة حرارة (-20 درجة مئوية) في الفريزر العميق لإجراء الدراسة النهائية للاختبار الإنزيمي المرتبط بالمناعة.

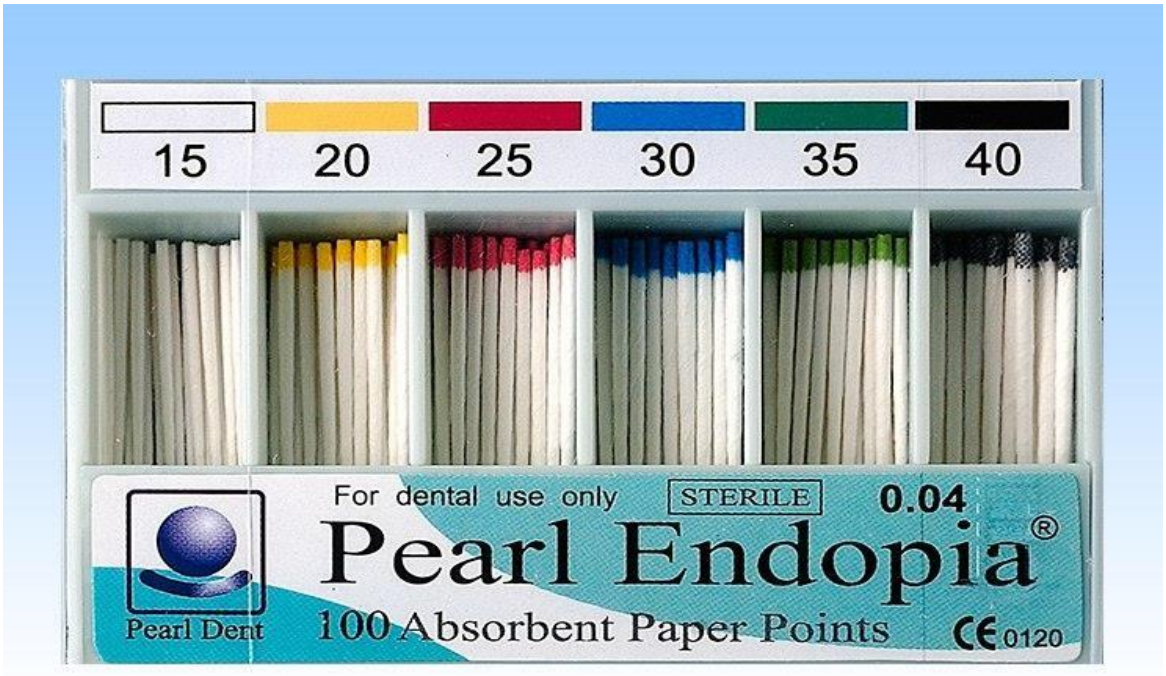
أظهرت سجلات هذه الدراسة نتائج إيجابية بنسبة (64%) في الكشف الجزيئي لبكتيريا عصوية المكورات ذات الصبغة الإيجابية. كما وقد أظهرت نسبة (58%) من العينات نتائج إيجابية لبكتيريا العصوائية الميلانينية ذات الصبغة السالبة ، وكذلك نسبة (36%) نتائج إيجابية لبكتيريا تانريلا فورساثيا ذات الصبغة السالبة .تم استخدام تقنية مقايسة الامتصاصية المناعية للانزيم المرتبط لغرض

قياس مستويات انترلوكين-8 وايضا" مستويات عامل النمو التحويلي نوع بيتا-1 في لعاب مرضى التهاب حوائط التاج.

كما وقد أظهرت النتائج أن تركيز إنترلوكين-8 وعامل النمو التحويلي بيتا-1 أعلى في مرضى التهاب حوائط التاج بالمقارنة مع مجموعة السيطرة وقد كان متوسط تركيز إنترلوكين-8 في لعاب المرضى هو (145.66±28.1)، بينما كانت مجموعة السيطرة هي (53.16±13.03)، وكان هنالك فروق ذات دلالة إحصائية عالية جدا بين مجموعة التهاب حوائط التاج ومجموعة السيطرة. كما وقد أظهرت مستويات عامل النمو التحولي بيتا-1 في اللعاب لمرضى التهاب حوائط التاج والمجموعة السيطرة فروقًا إحصائية عالية جدًا وقد كان الارتباط وثيق جدا" بين الانترلوكين-8 وعامل النمو التحويلي نوع بيتا-1 في مرضى التهاب حوائط التاج عن الاشخاص الغير مصابين.

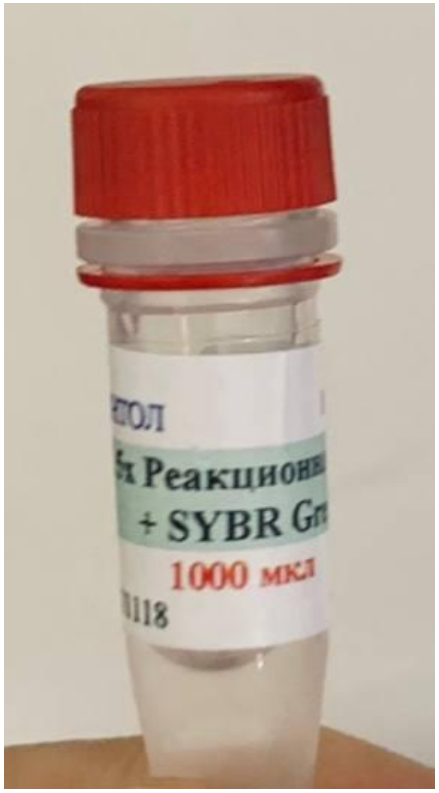


Periodontal instruments



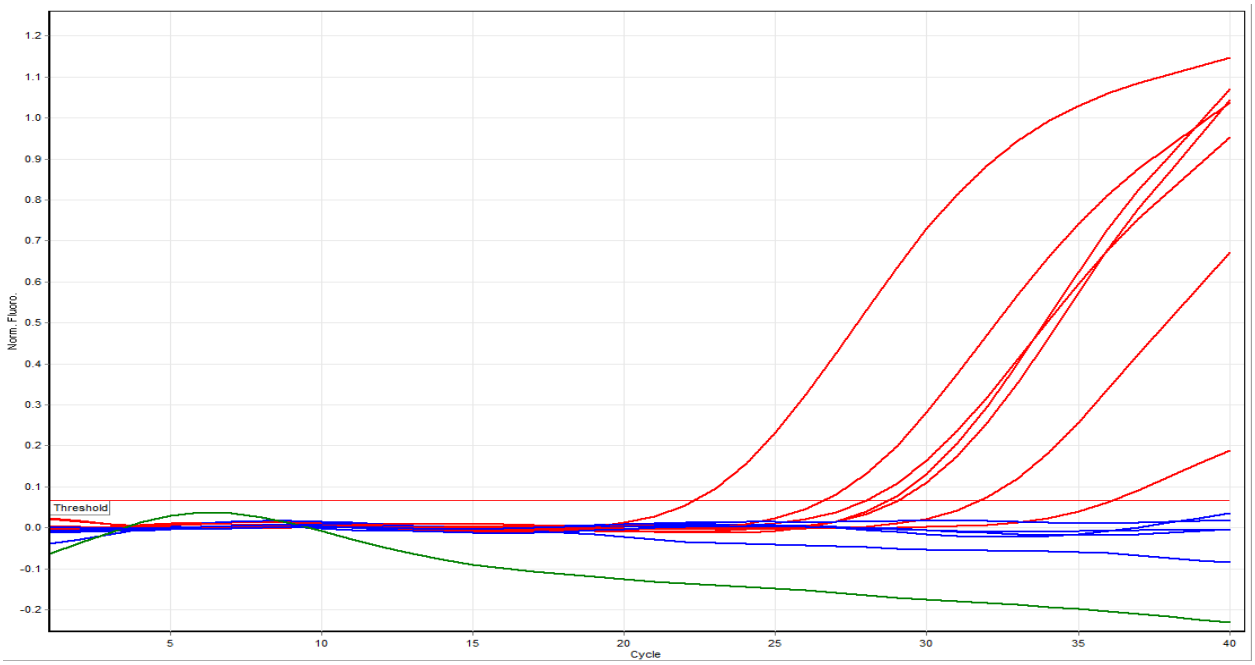
Paper Points

PCR device

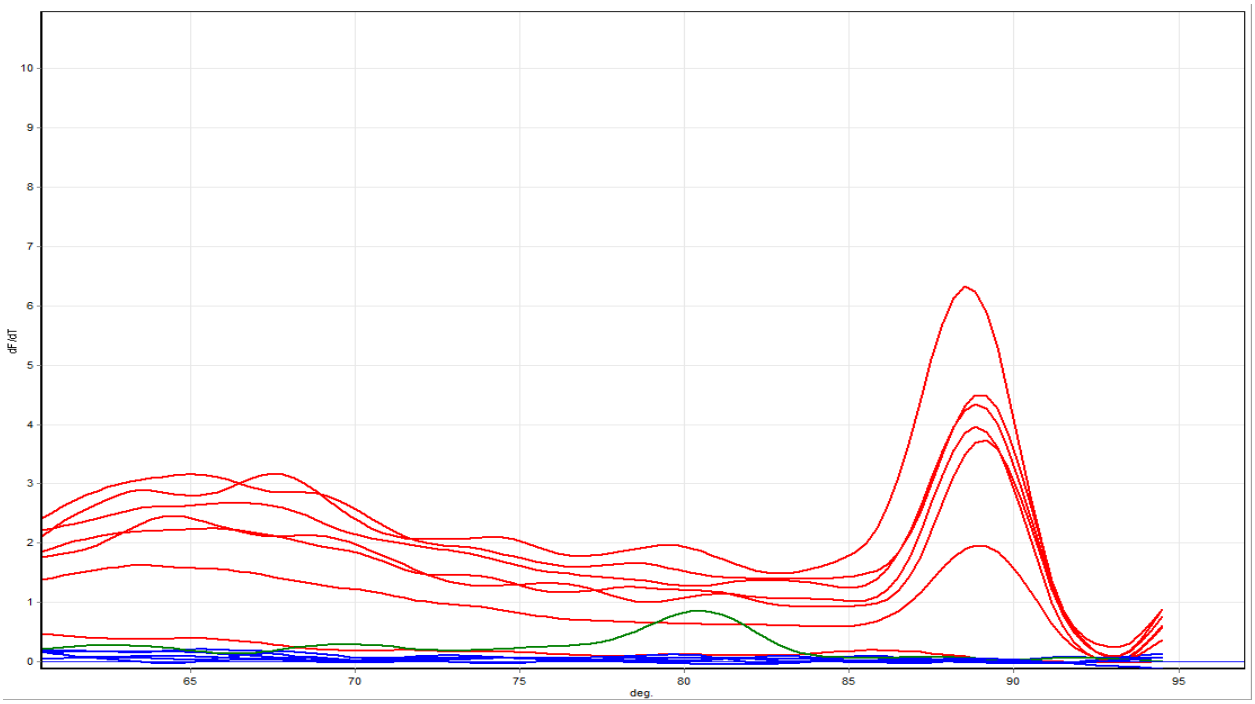


Syper Green

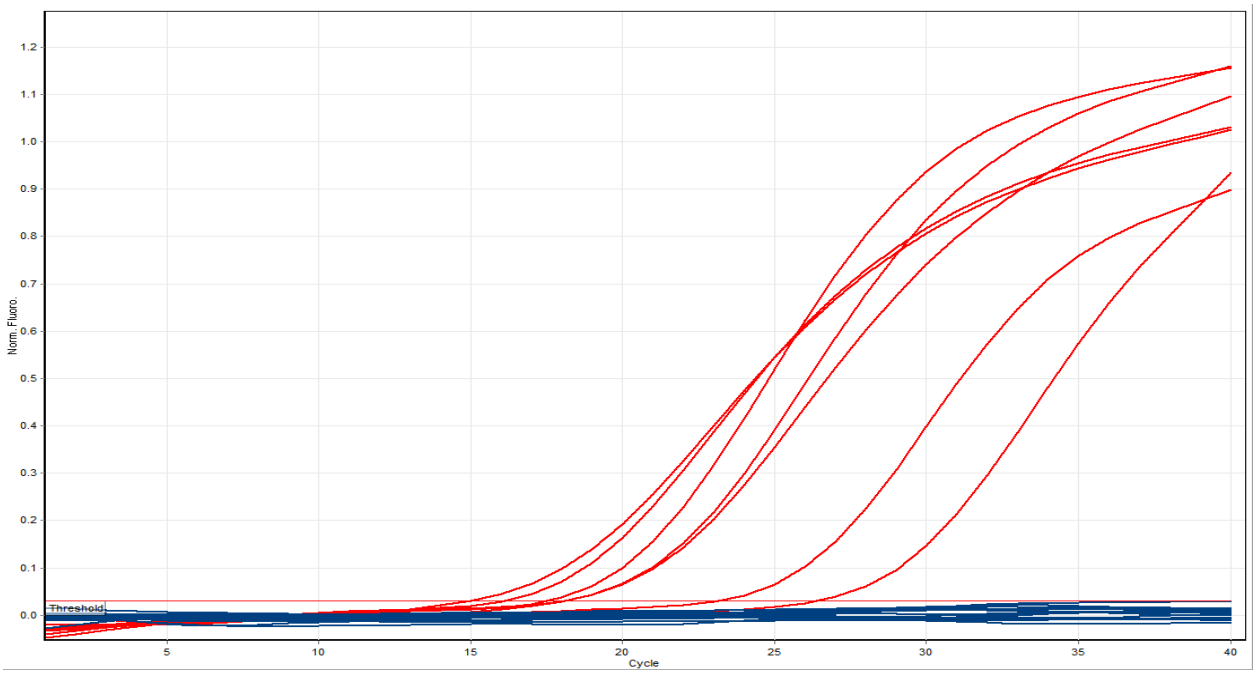
MgCl2



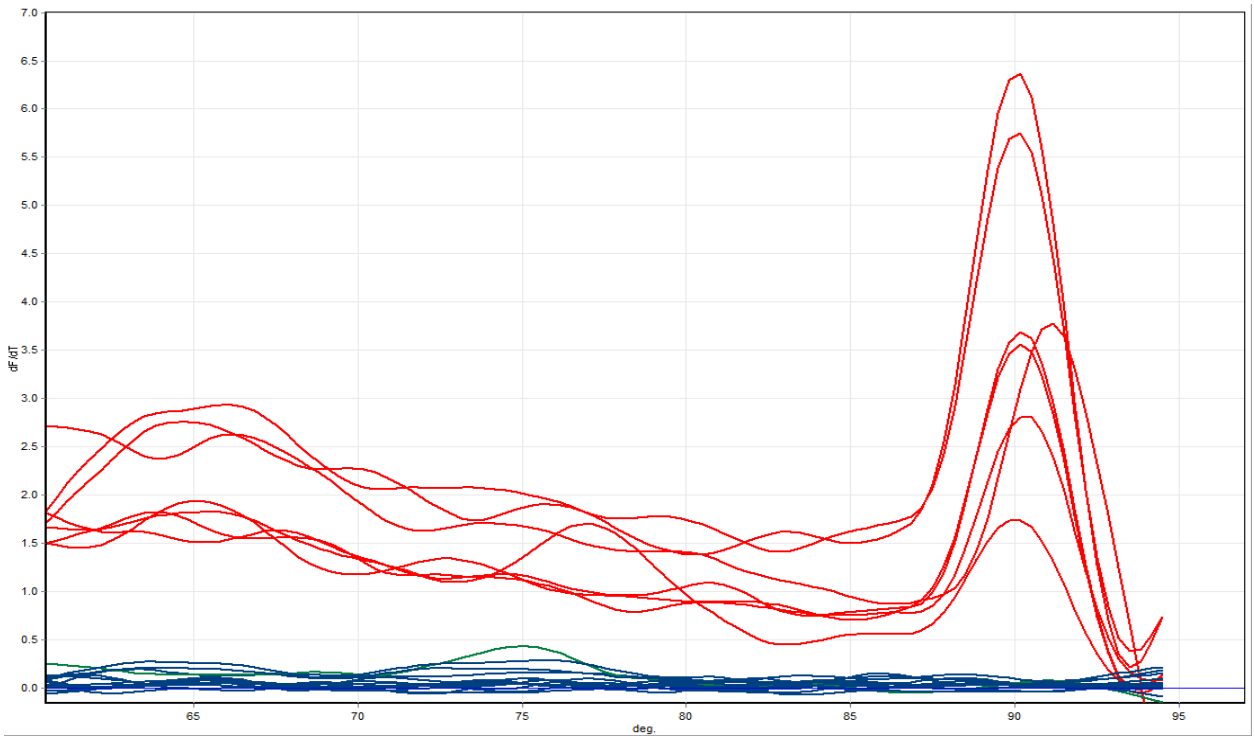
Amplification of *streptococcus mutans*



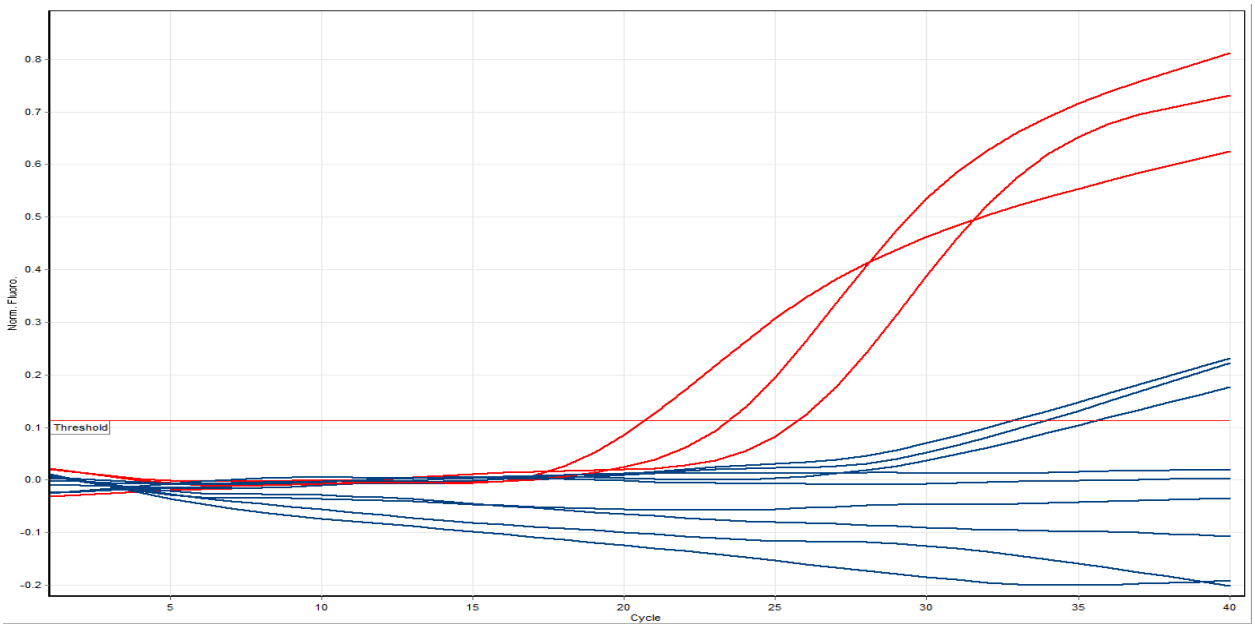
Melting of *streptococcus mutans*



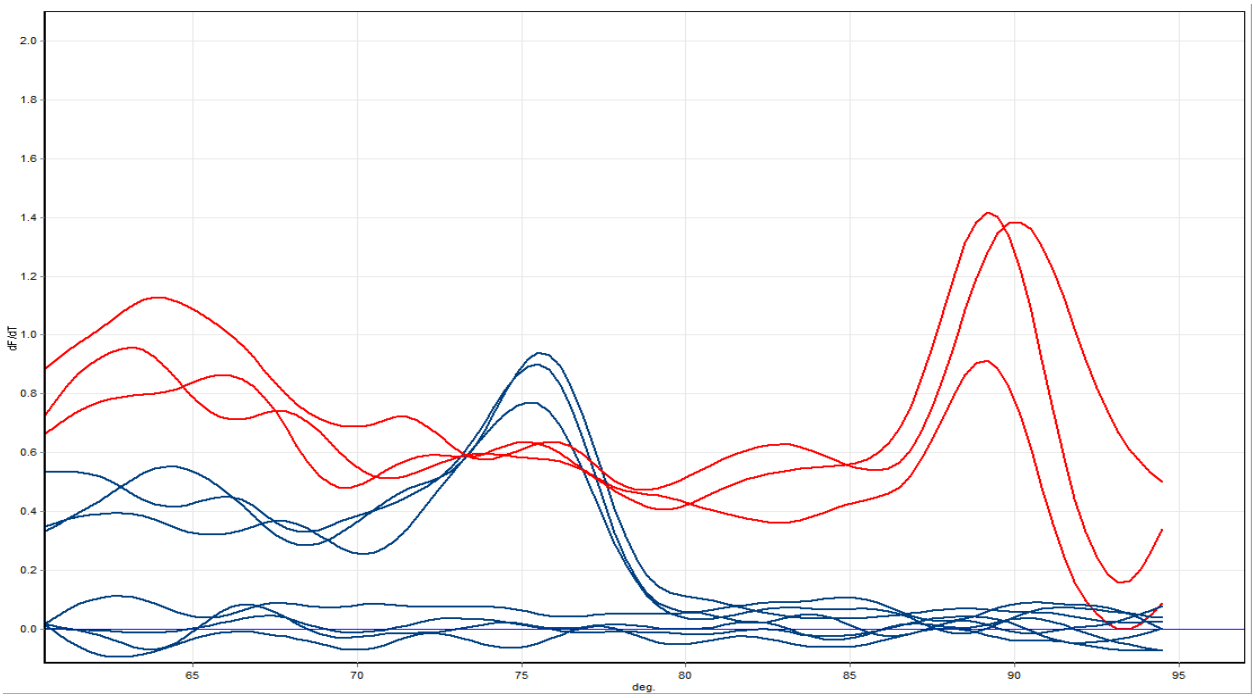
Amplification of *Prevotella intermedia*



Melting of *Prevotella intermedia*



Amplification of *Tannerella forsythia*



Melting of *Tannerella forsythia*

APPENDIX A

موافقة للاشتراك في البحث العلمي

اسم الباحث:

عنوان البحث:

مكان اجراء البحث:

انت مدعو للمشاركة في بحث علمي سريري سيجري في..... الرجاء ان تأخذ الوقت الكافي لقراءة المعلومات التالية بتأن قبل ان تقرر إذا كنت تريد المشاركة ام لا، بإمكانك طلب ايضاحات او معلومات اضافية عن اي شيء مذكر في هذه الاستمارة او عن الدراسة ككل من طبيبك.

في حال وافقت على المشاركة في هذه الدراسة سيبقى اسمك طي الكتمان لن يكون لأي شخص ما لم ينص على ذلك حق الاطلاع على ملفك الطبي باستثناء الطبيب المسؤول عن الدراسة او المعاينة.

موافقة مشترك

لقد قراءة استمارة القبول هذا و فهمت مضمونها، تمت الاجابة على جميع اسالتي وعليه فاني حرا مختارا اجيز اجراء هذا البحث وافق عليه واني اعلم الدكتور..... وزملاءه ومعاونيه او مساعديه سيكون مستعدين للإجابة على اسئلتي وانه باستطاعتي الاتصال بهم على الهاتف..... إذا شعرت لاحقا ان الاجوبة تحتاج الى مزيد من الايضاح فسوف اتصل بأحد اعضاء الاخلاقيات. كما اعرف تمام المعرفة بانني حر في الانسحاب من هذا البحث متى شئت بعد التوقيع على الموافقة دون ان يؤثر ذلك على العناية الطبية المقدمة لي.

اسم المشترك:

توقيع المشترك:

التاريخ:

Case sheet

Name:	
Age & Gender:	
Tooth No.:	
Chief Complain: **History of complaint:** **Medical history:** **Allergic to:** **Oral hygiene:** **Clinical examinations:** **Radiographic examinations:** **Diagnosis:** **Treatment planning:** **Case evaluation:**	

Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Babylon
College of Dentistry
Department of Microbiology

Microbiological Detection by Real Time PCR and Cytokins Assessment in Patients with Pericoronitis

A Thesis

Submitted to the Council of College of Dentistry / University of Babylon
in Partial Fulfillment of the Requirements for the
Degree of Master of Science in Oral and Medical Microbiology

By

Ahmed Nabeel Qasim

Supervised by

Professor
Dr.Bhaa Hamdi Hakeem

Professor
Dr.Suha Abd Alhussain Hendi

2023 A. D.

1444 A. H.

بسم الله الرحمن الرحيم

"نرْفَعُ دَرَجَاتٍ مِّن نَّشَاء وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ"

صدق الله العلي العظيم

سورة يوسف(76)

Supervisors Certification

We certify that this thesis entitled (**Microbiological Detection by Real Time PCR and Cytokins Assessment in Patients with Pericoronitis)** was prepared at the College of Dentistry, University of Babylon, as a partial requirement for the degree of master of science in oral and medical microbiology.

Signature

Professor

Dr.Bhaa Hamdi Hakeem

Signature

Professor

Dr. Suha Abd Alhusain Hendi

In view of the available recommendation, I forward this thesis for debate by the examination committee.

Signature

Assist. Prof.
Dr. Ahmed Mohammed Abbas

Head of the Department of Microbiology

College of Dentistry / Babylon University

/ / 2023

Dedication

To My wounded country and its martyrs and its army…

To my dear father and my dear mother…

To my wife and my daughters…

Fatima and Dania

To my teachers…

I appreciate your effort

I present this modest research

Ahmed Nabeel

Acknowledgments

First of all, I am indebted to Allah for allowing me to excel in my effort to complete this thesis, and for blessing me.

I am extremely grateful to my brilliant supervisors

Dr. BAHAA HAMDI AL-AMMEIDI

And

Dr. SUHA ABD ALHUSSAIN HENDI

For Providing All Required Resources for the Successful Completion of This Work and Encouragement all the time.

I'd like to extend my thanks to

Assist. Professor Dr. Ahmed Mohammed Abbas the Head of the Department of Microbiology.

Special thanks are also given to the Faculty of Dentistry / Babylon University and the Department of Microbiology for awarding me this opportunity to carry out my research.

I would like to extend my heartfelt thanks to all the patients who participated in this study

Finally,I wish to express my gratitude

To all my teachers who rendered their help during the period of my project work.

Ahmed Nabeel

Summary

The word "pericoronitis" refers to an inflammation of the soft tissues in the mouth caused by an infection of the gingiva and dental follicle, which are close to the crown of an immature tooth. It is commonly accepted that this process is accelerated by the accumulation of food particles near the opercula (the flap of gum tissue that partially covers an erupted tooth) and the occlusal force of the opposite tooth cause injury to the tissues in the pericoronal region. The study was done at the University of Babylon College of Dentistry and in the private clinic from January 2022 to June 2022. Fifty patients (n=50) have been diagnosed with pericoronitis, are (33) females and (17) males, and thirty (n=30) healthy adults serving as the study controls group. The age groups studied were above 16 years.

Swabs are collected by isolation of the lower third molar and dry area by air syringe then a sterile 3 paper point is inserted in pericoronal pockets until resistance felled for 30 seconds and then the paper point is put in an Eppendorf tube containing phosphate buffer solution and stored at (-20C) deep freeze until it is used for real-time PCR technique. Saliva collected, rinse the mouth with distilled water (10ml) for 30-60 seconds to ensure the removal of any debris. After 2 minutes of saliva accumulation in the patient's mouth, the patient was instructed to spit the collected saliva into the receiving vessel. Unstimulated saliva samples containing 2 ml are centrifuged for 15 minutes at 4000 rpm; the clear supernatant is eliminated, pipetted into plain tubes, and kept at (-20 C) in a deep freezer to make the final immunological examination study by the enzyme-linked immunosorbent assay (ELISA) test.

The results displayed about (64%) of the sample’s positive results in molecular detection for gram-positive *Streptococcus mutans.* Also showed that about (58%) of the samples had positive results for gram-negative *Prevotella intermedia* and (36%) positive for gram-negative *Tannerella forsythia*. The Immunological study used enzyme-linked immunosorbent assay (ELISA) to

determine Interleukin - 8 (IL-8) and Transforming growth factor beta - 1 (TGF-B1) concentration in the saliva of pericoronitis patients.

The result showed the concentration of Interleukin-8 and Transforming growth factor-beta 1 is higher in patients with pericoronitis infection than in control. The mean±S.D concentration of Interleukin-8 in saliva for patients is (145.66±28.1) while the control group is (53.16±13.03) which has a highly significant statistical (p-value 000) between pericoronitis and control group. The level of Transforming growth factor-beta 1 in the saliva of patients with pericoronitis and control groups highly significant statistics(p=000). The correlation of immune parameters Interleukin-8 and transforming growth factor-beta 1 is visible in pericoronitis patients through the strong correlation among them (p=000).

List of Contents

List of table

List of Figures

Abbreviation

bp	Base pair
CT	Cycle threshold
°C	Degrees centigrade
DNA	Deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
EDTA	Ethylene Diamine Tetra acetic acid
F	Forward
IL-8	Interleukin -8
µl	Micro liter
µm	micrometer
NFW	Nuclease free water
N	Number
OD	Optical density
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
P-value	Probability value
R	Reversed
RT-PCR	Real-time polymerase chain reaction
TGF-B1	Transforming growth factor- beta 1

1. Introduction

1.1. Pericoronitis

Pericoronitis is a term used to describe inflammation of the soft tissues surrounding the crown of a tooth that is erupting or has an incomplete eruption(Schmidt *et al.*, 2021). The condition is most frequently observed in late adolescence or early adulthood, and a number of variables are thought to have contributed to its development.

Under conditions of low immune resistance (stress, viral recovery), the oral microflora may acquire a pathological potential and aid in the presentation of symptoms. (Bradshaw *et al.*, 2012). This area is covered by gingiva, and food debris lodges beneath the covering gingiva, which is then invaded by bacteria (Scheinfeld *et al.*, 2012; Mardini & Gohel, 2018).

Pericoronitis is a polymicrobial mixed infectious periodontal pathology(Stedman, 2005; Dodson *et al.*, 2012). The bacteria that develop in the distal pseudo-pocket are the primary source of pericoronal tissue inflammation and occlusal damage caused by the opposing tooth. The identification of the microbiota of the pericoronitis area is critical in the medical treatment plane (Djohan *et al*., 2022). In a pseudo pocket, the microbial species predominately grows in anaerobic conditions(Sedghi *et al.*, 2021). Numerous possible bacteria have been mentioned in earlier reports, including various Streptococcus as well as various types of Staphylococcus, Fusobacterium, Prevotella, and Veillonella, Porphyromonas gingivalis, and it also Parvimonas Micra (previously known as Peptostreptococcus micros and Micromonas micros), Tannerella forsythia(Colombo & Tanner, 2019).

Yousefi *et al.* (2020) demonstrated that the multimicrobial, primarily anaerobic bacteria that cause pericoronitis are similar to those that cause gingivitis or periodontitis (Yousefi *et al.*, 2020). Cultivation studies can drastically underestimate

microbial diversity because many of microbes cannot be cultured using standard techniques, and the bacteria that cause pericoronitis are mostly Gram -negative bacteria that require anaerobic growth conditions (Jervøe-Storm *et al.*, 2005). Polymerase chain reaction (PCR) has become known as an essential instrument for the quickly, sensitive, and specific identification of bacterial pathogens in third molar pericoronitis(Induri *et al.*, 2021). Due to the advantages of PCR, it has been widely used for both diagnosing and identifying bacterial species(Ma *et al.*, 2021).

Interlukin-8 , a chemokine family member, was initially recognized by Matsushima and Oppenheim(Matsushima *et al.*, 2022). It is the most significant human neutrophil chemoattractant and activator, as well as an essential mediator for granulocyte buildup(Watanabe *et al.*, 2019). In pericoronitis, IL-8 plays a role in the beginning and amplification of acute inflammatory responses as well as persistent inflammatory processes. Interleukins and also colony-stimulating factors, growth factors, and cytotoxic factors are among the many types of soluble mediators known as cytokines. The cellular processes of differentiation, proliferation, regeneration, development, repair, inflammation, and homeostasis are all significantly influenced by cytokines. In the damaging inflammation imposed on by microbes, pro-inflammatory cytokines are crucial, which leads to the onset of the periodontal pathogen in the pericoronal area. (Morandini *et al.*, 2011) .Anti-inflammatory cytokines are a class of immune-regulatory molecules that regulate pro-inflammatory cytokine secretion, reducing inflammation and promoting healing. In addition, the level of anti-inflammatory cytokines has increased (Herder *et al.*, 2013).

Transforming growth factor-beta 1 is a critical cytokine with pleiotropic characteristics that regulates the inflammatory infiltrate by having two properties which are pro-inflammatory and anti-inflammatory as it brings neutrophils, macrophages, mast cells, and lymphocytes, as demonstrated in a number of studies

(Heidari *et al.*, 2013). It also leads these cells to release proinflammatory substances such as interleukin -1 (IL-1), interleukin -6 (IL-6), and tumor necrosis factor -a(TNF-a). It also inhibits cell growth (Wilson *et al.*, 2022). TGF-1 is a charming protein for studying in the pathology of pericoronitis due to the characteristics listed above.

The effect of the inflammatory response is determined by a sight balance between pro and anti-inflammatory cytokines, according to findings from various cytokine studies(Zhong *et al.*, 2020). If a bone is destroyed during pericoronitis, it has a significant effect on bone regeneration. Chemotaxis, proliferation, and differentiation occur concerning in focused osteoblasts, making it a potent osteoblastic bone formation stimulator(Hatami Kia *et al.*, 2018).

1.2 Aims of the Study

Investigate the most common bacteria and the role of IL-8 and TGF-beta-1 in pathogenicity of pericoronitis, so to achieve this goal many approaches were adopted by

1. Detecting of predominant bacteria in pericoronitis patients by using real-time PCR.
2. Determine concentration of IL-8 and TGF-B-1 in saliva by using the ELISA technique and study the relationship between IL-8 and TGF-beta-1 in pericoronitis patients.

2. Literature Review

2.1 Features of the gingiva

Gingiva is part of oral mucosa which surrounds the necks of the tooth and covers the alveolar ridge. It is a part of the periodontium's tooth-supporting structure connecting with the tooth via the gingival sulcus, it protects the underlying tissues of the tooth attachment from the oral environment and gingival epithelium acts as a protector to the deep structure and allows a selective exchange with the oral environment(Al-Shabeeb *et al.*, 2019).

The gingiva can be classified into three types:1- interdental. 2-marginal unattached. 3-attached gingiva. There is a central base of connective tissue in the gingiva that is enclosed by stratified squamous epithelium (sulcular epithelium, junctional epithelium, and oral epithelium) and the underlying connective tissues termed lamina properia (Reddy, 2017). The gingival epithelium provides protection for the underlying structures while permitting only limited exchange with the oral environment. Keratinocytes proliferation, differentiation, and apoptosis in order to carry out this function. In gingiva cells, there are several layers that go by the names “stratum basale, stratum spinosum, stratum granulosum, and stratum corneum” are all present, as well as a keratin layer containing keratinized cells. (Newman *et al.*, 2006). Cells flatten as intracellular connections increase in response to epithelial flow and nuclei disappear which is the most obvious morphologic change(Jia *et al.*, 2022).

The size and number of the oral epithelium cells are continuously renewed and the ratio of newly formed cells to those that are shed keeps the thickness of gingiva renewal constant in terms of both size and number. The gingiva's thickness is

maintained by a balance between the formation of new cells and the shedding of old ones(Yang *et al.*, 2019). The connective tissue of gingiva underlying the epithelium, the lamina propria, contains a lot of extracellular collagen but few cells. The epithelium layer, on the other hand, lacks the presence of cells. About 5% of gingival connective tissue is generated up of cells, including mast cells, neutrophils, fibroblasts, immune-inflammatory cells, and lymphocytes. (Beklen Tanzer, 2010).

The connective tissue close to the base of the sulcus contains lymphocytes in healthy gingiva. Neutrophils can also be noticed in the sulcus and periodontal fibrous tissue(Medara *et al.*, 2021). Marginal gingiva's connective tissue is made of dense collagen and has a noticeable system of collagen fiber networks known as gingival fibers. They are made of collagen type-1 and function to tightly attach the marginal gingiva to the tooth (Park, 2022)

2.2 Pericoronitis

The term pericoronitis was first introduced into the dental literature by Bloch in 1921(Malhotra and Kaur, 2012).It common term for the swelling of the soft tissues surrounding the dental crown in a lower third molar that is only partially erupted (Katsarou *et al.*, 2019).

In clinical terms, Pericoronitis is also known as operculitis (Douglass & Douglass, 2003). When the eruption is delayed or prevented, most frequently because of malposition or impaction, classic pericoronitis is likely to develop. Consequently, pericoronal inflammation only occasionally occurs in connection with other impacted teeth, such as the upper third molar, and is almost always linked to the mandibular third molar, which is the tooth that experiences impaction most frequently. The third molar's pseudo-pocket, which is the zone between the crown of tooth and follicle, develops around it and collects bacterial plaque beneath the soft

tissue cap, predisposing it to inflammatory complications (Huang *et al.*, 2020; Schmidt *et al.*, 2021).

A diagnosis of acute, subacute, or chronic pericoronitis was made based on the clinical presentation. Early-stage pericoronitis patients frequently describe pain, intraoral swelling, redness, mucosal ulceration, and loss of function(Schalch *et al.*, 2019). If treated properly and early enough, it can recover easily, quickly, and cheaply without the use of systemic antibiotics(Schmidt *et al.*, 2021). Antibiotics should only be used in severe cases where an infection is spreading and systemic symptoms are present.(Wehr *et al.*, 2019). due to the possibility of resistance developing (Palmer, 2016). On the other hand, a significant number of dentists routinely recommend unnecessary antibiotics for pericoronitis(Koyuncuoglu *et al.*, 2017). The challenge is the absence of a standardized, evidence-based treatment for initial pericoronitis(Schmidt *et al.*, 2021). The development of the initial condition is well known to cause lymphadenopathy, fever, malaise, palatoglossal arch asymmetry, difficulty swallowing, and trismus, and can end in a life-threatening condition known as Ludwig's angina(Mayor Hernández *et al.*, 2011).

Since pericoronitis is caused by an overgrowth of bacteria in the difficult-to-clean areas that form during third molar eruptions, maintaining good oral hygiene is important for avoiding the condition (Caymaz and Buhara., 2021). The use of mouthwash, floss, and proper tooth brushing techniques can reduce the amount of bacteria at the eruption site. However, some areas might not be reached or cleaned during routine oral hygiene. To help patients recognize the symptoms and signs of pericoronitis and seek dental care, patient education is crucial(Kwon and Serra., 2022). Eliminating a potential infection source is another way to prevent pericoronitis from developing. It is reasonable to think about prophylactic third molar extractions if, after radiographic examination, it is determined that a patient's

third molar has a poor prognosis to erupt into functional occlusion, increasing the risk of developing pericoronitis. The incidence of pericoronitis is 4.92% frequency among patients between the ages of 20 and 25, and the lower third molar is involved in 95% of cases(Katsarou *et al.*, 2019).

2.3. Classification

Pericoronitis can be divided in relation to the process of tooth eruption:

I. Transient— happens during the eruption of tooth.

II. Non - transient— following the cessation of tooth growth.

and divided in relation to the development:

a. Acute - sudden onset with sever symptoms.
b. Chronic - long-lasting, mild, or asymptomatic.

Dentitio difficilis is another name for pericoronitis. However, this word typically refers to any issue with difficult teeth eruption, so it should be understood as term for pericoronitis. (Schmidt *et al.*, 2021)

2.4. Prognosis

Pericoronitis has an appositive relationship with tooth plaque accumulation(Caymaz & Buhara, 2021). The pericoronitis may go away after the eruption is finished if the third molars have enough room to grow into a cleansable position. However, if a tooth does not erupt into a suitable location, pericoronitis may continue or return. The third molars should be extracted in cases of such symptomatic pericoronitis to relieve symptoms and enhance quality of life. Additionally, it has been demonstrated that having symptomatic third molars extracted helps the neighboring second molars' periodontal health(Ashley *et al.*, 2015).

2.5. Clinical Manifestation and Diagnosis

Pain, redness, swelling, heat, and functional loss are some of the inflammation symptoms that are expressed by pericoronitis. Localized pain that is only felt in the soft tissues surrounding the erupting teeth starts out. Patients frequently characterize it as pulsating, ultimately spreading to nearby tissues or faraway locations (soft palate, mouth floor, retromandibular and submandibular space, throat, ear, or temporomandibular joint)(Chi *et al.*, 2017). When the load is given to the affected region, it typically gets worse over time and becomes visible. In addition to impairing sleeping, pain's increase during mastication may cause one to eat less. During the physical evaluation, soft tissue above and around a molar is discovered to be swollen and reddened Figure (2-1).

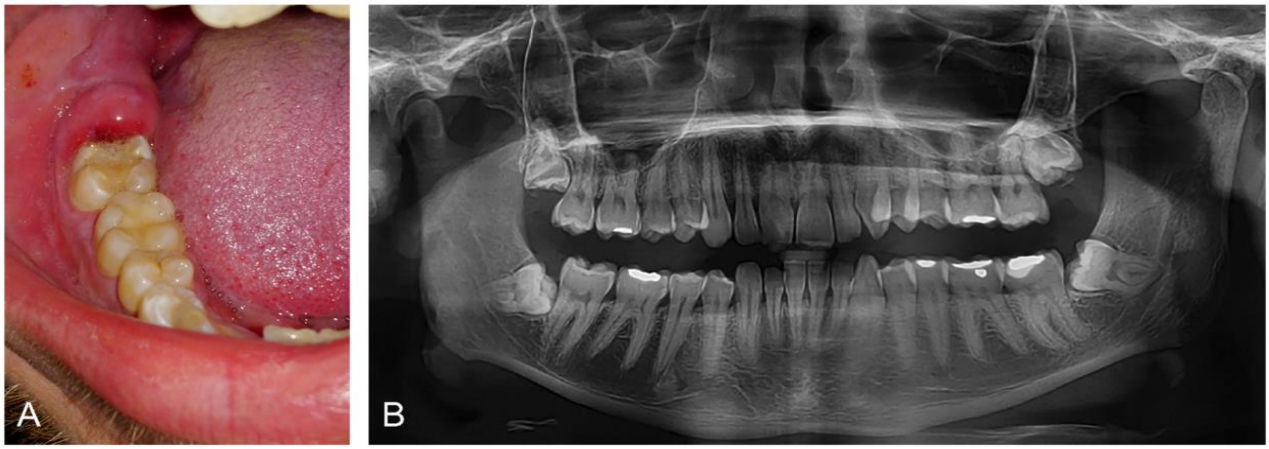


Figure (2-1) Pericoronitis clinical symptom and panoramic scan. Image (A) shows inflamed soft tissues surrounding the right lower third tooth that has not fully emerged. A prior panoramic scan of the same patient is shown in Image (B), showing third teeth that have not fully emerged (Schmidt, *et al* 2021).

Decomposing tissue and debris can lead to bad odor (halitosis), poor taste, or changes in how one feels smells(Yurttutan *et al.*, 2020). Regional lymphadenopathy of the neck and mandible typically affects one side. Symptoms of a more serious course, such as infection spreading to nearby tissue spaces, such as the deep spaces of the head and neck include bilateral lymphadenopathy, pyrexia, palatoglossal arch asymmetry, facial asymmetry, malaise, difficulty swallowing "dysphagia," or a restriction in mouth opening that may be accompanied by pain "trismus" are all symptoms of a disorder(Wray *et al.*, 2003; Chi *et al.*, 2017).

Clinical examination is used to make the diagnosis of pericoronitis fixed on the appearance of the inflammation of soft tissue connected to the partly erupted teeth. The following conditions should be taken into consideration during alternative diagnosis: Dental cavities, Gingivitis, mucous issues, pulpitis, pulp necrosis, periapical abscess, food packing, alveolar osteitis “dry socket”, peritonsillar abscess,

pterygomandibular cavity abscess, TMJ disorders, and myofascial pain are all examples of oral infections.

Pericoronitis, like any other bacterial infection, results in the synthesis of mucus. If it is not cleared, it expands up and causes a cyst in the peri coronal cavity. The fluid expands as it accumulates. Ligaments and performed anatomical spaces in the sublingual space, submandibular space, parapharyngeal space, pterygomandibular space, infratemporal space, sub masseteric space, and buccal space aid in the spread of an infection into the neighboring regions(Dhonge *et al.*, 2015). Pus accumulation behind the tonsil causes Quinsy, another name for the peritonsillar abscess. Its signs include temperature, lymphadenopathy , throat discomfort, dysphagia , dyspnea, vocal change, and palatal arch irregularity due to pus accumulation(Basson & Kilborn, 2010). Pus elimination, medicines, enough water, and painkillers are used in treatment (Galioto, 2017). Partnership of the sublingual, submandibular, and submental areas may result in Ludwig's angina, which are a potentially fatal disease. Swelling concerning the lower jaw and upper neck, temperature, lymphadenopathy, throat discomfort, dysphagia, dyspnea, raise of the mouth floor, and tongue protrusion are all signs of a phlegmonous illness (Candamourty *et al.*, 2012). It appears suddenly and spreads quickly with a chance of obstructing the airways. Early detection and intervention are therefore crucial. The same concepts are used for treatment: fluid replacement, antibiotics, and pain relief (Chou *et al.*, 2007). Resolving the main cause of illness is a necessary component of the successful therapy of these complications.

2.6. Etiopathogenesis

Even so, pericoronitis is a bacterial infectious disease, its cause is largely defined by local morphological factors rather than the transmission of the infectious agent. The majority of the microorganisms implicated are obligate and facultative anaerobes, like *Propionibacterium spp.*, *Prevotella*, *Veillonella*, *Tannerella forsythia*, and *streptococcus* (Djohan *et al*., 2022). .These bacteria are considered and found in the normal flora of the oral cavity(Aas *et al.*, 2005), Hence, the issue isn't the actual presence of these microbes, but rather their accumulation, overgrowth, and careless management in the small space between the soft tissue and the crown of the tooth. Figure (2-2).

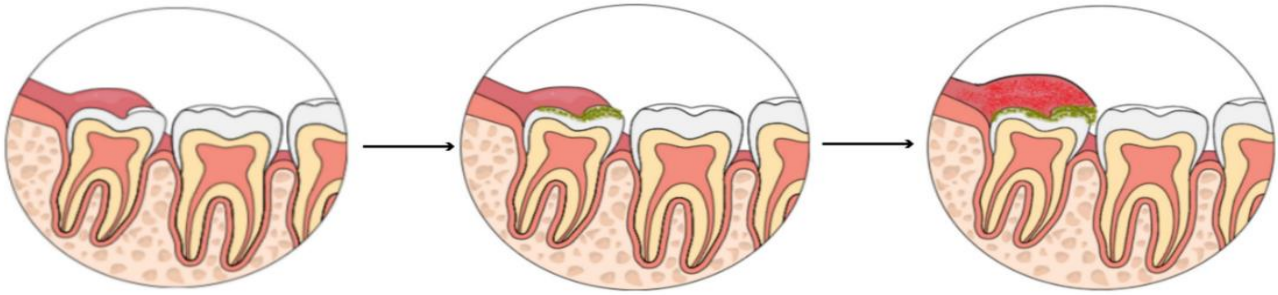


Figure (2-2) Pericoronitis development – scheme for illustration. Images show a soft tissue-covered partly erupted third tooth. Soft tissue inflammation is caused by plaque and dietary stasis (green) (Schmidt, *et al* 2021).

The form or size of the pocket and its hole, as well as the flap's anatomy, and the rotation and position of the tooth all have a major effect on how pericoronitis develops (McArdle *et al.*, 2018; P. Singh *et al.*, 2018) . We identify variables that

contribute to the prevalence and intensity of pericoronitis, in addition to the circumstances needed for its production, such as an erupted tooth and bacterial development. They are either limited or widespread. Soft tissue damage, neglect mouth hygiene, and trapping of foreign substance are the most prevalent local causes. When the pericoronal flap is harmed by food particles or the straight bite of the opposing teeth, it can be injured during mastication. Inadequate dental care with poor oral hygiene can also contribute to trauma (Chen *et al*., 2021).

Inadequate dental hygiene causes plaque to accumulate around the flap, contributing to irritation or bacterial buildup beneath it. Food remnants are one of the most prevalent foreign particles that become held between the soft tissues and the crown's tooth. Their subsequent decomposition promotes the growth of microbes and results in an inflammatory response in the tissue. Systemic variables that promote the expansion of pericoronitis or exacerbate its progression include any illness or condition that impairs the immune system or wound healing. These elements can be either transient or lasting. Temporary causes include emotional and physical tension, as well as upper respiratory tract infections(Timmerman and Parashos, 2020). The researcher found that changes in the seasons in respiratory infection epidemics linked to height in the number of patients with pericoronitis, and 33% of pericoronitis patients confessed to a prior upper respiratory illness. Bataineh *et al*. came to very identical findings, stating that the most important risk factor for pericoronitis was an upper respiratory illness (37.9%).(Bataineh and Al Qudah, 2003).

Likewise, a research by Meurman *et al*. revealed a substantial rise in the prevalence of respiratory tract infection in the two weeks prior to acute pericoronitis, with the incidence peaking three days prior to pericoronitis(Meurman *et al.*, 1995). Stress was also found to play a prominent part in pericoronitis, occurring prior to it

in 17–66% of case (Dalgıç *et al.*, 2022). Fortunately there have been no mechanistic studies on the role of stress in the development of pericoronitis, the general immune system alterations caused by stress may contribute to the development of pericoronitis (Dhabhar, 2014). In addition, an intriguing connection to menstruation was discovered. Concurrent menstruation was reported by 4-12% of women with pericoronitis (Bataineh & Al Qudah, 2003; Almutairi, 2019).

Furthermore, Pericoronitis affected more than half of the females within a short period of time of their menstruation cycle(Almutairi, 2019). The authors suggested a hypothesis that the emotional component might represent the most interesting role, as most of these women confessed to having premenstrual symptoms. Both psychological tension and immune modulation during the premenstrual period could contribute to the progression of pericoronitis. Diabetes mellitus and immune deficiency diseases are examples of persistent systemic variables. If pericoronitis develops as a complication of another disease, it may be classified as an opportunistic infection due to a compromised immune system. Furthermore, users are found to have a higher incidence of pericoronitis (R. Singh *et al.*, 2020). According to some research, participation in serious sports is a risk factor for pericoronitis, which has a documented incidence of 5-39% in sports benefits (Ashley *et al.*, 2015; Tripodi *et al.*, 2021). The writers attribute the reason to the powerful players' nutritional, hygienic, and behavioral habits rather than exercise. Systemic and local variables may co-occur and interact closely. The patient's immune system and mental health are both stressed by conditions like a recurrent upper respiratory illness. When sick, eating habits and dental hygiene requirements can decline, which can cause plaque to build up in the mouth. The chances that pericoronitis will occur is increased by all of these variables. Causes and risk factors are summarized in Table (2-1)

Table (2-1). Causes and risk factors for pericoronitis(Schmidt *et al.*, 2021).

Causes	Risk Factors	
	Local	Systemic
Imperfectly erupted tooth Bacterial accumulation	Pericoronitis in anamnesis Poor oral hygiene and plaque retention Traumatization of pericoronal soft tissues Debris entrapment	Upper respiratory tract infection Mental or physical stress Diseases impairing the immune system or wound healing (diabetes mellitus) Premenstrual phase Smoking

2.7. Immune Response

2.7.1. Innate and adaptive immunity

Pericoronitis is brought on by oral microbes suppressing epithelial cells, which leads to innate and adaptive immunological reactions as a result of infection. These procedures crush the tooth's supporting and encircling tissues, which eventually lead to bone and tissue loss. Periodontal diseases have been shown to start as a result of the bacterial biofilm (Lim *et al.*, 2020). According to the majority of research, the percentage of the damage caused by this disease is caused by the host's reaction rather than the germs themselves. The acquired immune reaction is formed after the natural immune response. Leucocytes are responsible for the innate immune reaction, which relies on pattern detection. “Toll-like” receptors are among the proteins present in these cells. As a result, any molecular unit that attaches to these receptors is identified as "foreign" and causes reaction from the host. Inflammatory

molecules, such as cytokines, are produced as a result of this reaction. Cytokines such as; TNF-a, IL-1, IL-6, and IL-8 Involve the activation of numerous cellular processes, such as phagocytic cell movement to the geographic distribution of the infection, which together make up the innate immune response (Kumar *et al.*, 2022).

Leukocytes in the innate immune system, which include natural killer cells, mast cells, eosinophils, basophils, and phagocytic cells like macrophages, neutrophils, and dendritic cells, find and eliminate organisms that could harm a person(Szollosi and Mathias, 2020). The acquired (adaptive) immune reaction comes after the innate immune response. The acquired immune reaction is strongly customized toward a specific organism. The acquired immune system remembers the infectious agent and can avoid it from producing illness in the future (Tomalka *et al.*, 2022).

So, specifically and remembering are two important characteristics of the adaptive immune response. An individual lymphocyte is a particle that makes up the adaptive immune system. The two main classes of lymphocytes are B cells and T cells, which can identify particular diseases with specificity. The microbes are killed by these cells' binding to them. Prior to being triggered, B cells and T cells “T-helper, T-cytotoxic, and T-regulatory” are identical and generated from the same stem cells. Compared to T-cells, which are closely associated with cell-mediated immune reactions, B cells play an important role in the humoral immune response cells release antibodies that fight external bacteria and their byproducts. T cells can be activated in a variety of ways, such as regulating B lymphocyte growth and antibody production, working with phagocytic cells to aid them eliminate viruses they have embraced, and recognizing and eliminating virus-infected cells(Moisa and Kolesanova, 2012).

2.7.2. cytokine

The beginning of the immune response is triggered by bacteria and their products, which accumulate in the space between the flap and tooth surface and mediate the destruction of connective tissue, while a large number of inflammatory mediators (cytokines and chemokines) are produced by numerous cellular components such as mast cells, dendritic cells, and macrophages. These cytokines are known as cytokine networks when they cooperate to modulate cellular actions and share the innate immune system(Kinane *et al.*, 2011). Although bacteria in the plaque are necessary in the starting of pericoronitis disease process, they did not always the reason for the real destruction of tissue that follows. The word "cytokine" is Greek in origin. ("cyto" – cell, as well as "kinos" – movement). They perform a number of tasks, such as the start, differentiation, and management of inflammatory and immune reactions, as well as the control of cell proliferation They engage with their receptors on the cell membrane to initiate biological activities. These chemicals are released by the host's living cells as paracrine or autocrine messages to attract immune system cells (chemokines), trigger inflammation (pro-inflammatory cytokines), or regulate inflammatory reactions (anti-inflammatory cytokines)(Arango Duque and Descoteaux, 2014). Immune cells respond to a disease by releasing cytokines during inflammation, which boosts immune cell activation and recruitment(Ferreira *et al.*, 2021). According to their function in the inflammatory process, cytokines could be categorized as pro-inflammatory, anti-inflammatory or both. The formation of additional cytokines, an increase in surface receptors for additional substances, the manufacturing of proteinase, or the reaction being blocked by a feedback mechanism can all result from the upregulation and/or downregulation of transcription elements and chemokine genes. The majority of cytokines generated during inflammation are made by inflammatory cells like

neutrophils, lymphocytes, and monocytes/macrophages. However, numerous local cells, including fibroblasts, epithelium cells, and endothelial cells, also generate them(Franklin, 2021).

2.7.2.A. Interleukin -8

Interleukin 8 is a primary regulatory cytokine in inflammatory processes. The regulating action of IL-8 on neutrophil entry into the tissue and its impact on capillaries have been demonstrated using high-affinity neutralizing anti-IL8 antibodies(Kotb & Calandra, 2003). Human IL-8 receptors are classified into two types: 351 and 360 amino acids, respectively, distinguish type A from type B. With seven transmembrane regions and a high degree of amino acid sequence similarity to other members of its family of G-protein-coupled receptors, IL-8 is a member of this group)Smit *et al.*, 2003). Neutrophils are the first line of defense cells against bacterial invading. It functions as a powerful angiogenic component as well as a chemoattractant. For neutrophils, IL-8 is a potent chemotactic protein. The cytokine IL-8, which is produced by macrophages and endothelial cells, attracts neutrophils, leading them to move into regions where they are needed, particularly in situations where there is inflammation and infection(Darif *et al.*, 2021).

IL-8 is a member of the interleukin-8 supergene family, which also contains short peptides with chemotactic action for particular populations of leukocytes(Hub *et al.*, 2020). This cytokine is produced and released by a variety of cells, including macrophages, lymphocytes fibroblasts, epithelial, and endothelial cells as well as by synovial cells(Tipton *et al.*, 2016). In inflamed areas, IL-8 pulls and stimulates polymorph nuclear leukocytes (PMN) it induces the adhesion of PMN to endothelial cells and their trans endothelial migration as well as the release of granule enzymes from these cells(Poplimont, 2020).

2.7.2.B. Transforming growth factor-B1

TGF is a multifunctional cytokine that controls proliferation, cellular differentiation, apoptosis, and survival in varied cell types with immune reactions and angiogenesis (Heidari *et al.*, 2013; Yoshimoto *et al.*, 2015). On normal cells as well as cells that are in the early phases of oncogenesis, it has an anti-proliferative damage. The TGF-signaling system looked to be functionally inactivated in cancer cells, which renders the usual modulation of cell proliferation and apoptosis ineffective(Bierie & Moses, 2006). Many different kinds of cells use TGF-B to synthesize connective tissue components like collagen, glycosaminoglycan, proteoglycan, fibronectin, and osteonectin (Huang *et al.*, 2015). Studies have demonstrated that TGF-B can be produced by all types of healthy cells, including inflammation cells, arterial endothelial cells, gingival fibroblasts, and epithelium cells (Yoshimoto *et al.*, 2015). The most effective development suppressor for fibroblasts, neural cells, lymphocytes, hepatocytes, epithelial cells, and endothelial cells is transforming growth factor (TGF)(Huang *et al.*, 2015). TGF-B1, the cytokine I'm referring to, appears to be correlated with the source of bone cells and environmental variables, and it has a significant effect on bone formation (Okada & Murakami, 1998; Raja *et al.*, 2009). The TGF-B1 is a dimeric polypeptide with a molecular weight of 2500 kDa that consists of two strands of amino acids joined together by disulfide bonds (Raja *et al.*, 2009). Mammals have been found to produce all three of the TGF- isoforms (TGF-1, TGF-2, and TGF-3), with TGF-1 being the most prevalent and widespread(Raja *et al.*, 2009; Heidari *et al.*, 2013). These isotypes share numerous biological functions that are typically essentially identical and are encoded by various genes(Wang *et al.*, 2022). It may have inhibitory properties and play a down-regulatory role in the production of some cytokines, including interleukin-1, metalloproteinase, and tumor necrosis factor-

alpha (Atilla *et al.*, 2006). Additionally, TGF-1 mRNA expression is likely found in the regulating T cells of gingival tissue and is linked to a greater risk for systemic diseases, such as rheumatoid arthritis and cardiovascular diseases, which are connected to periodontal illnesses(Atilla *et al.*, 2006) and this cytokine are associated with periodontitis patients (Hassan and alamid *et al.*, 2019). Transforming Growth Factor is thought to play both therapeutic and pathological functions because it is found in both early and late phases of periodontitis(Yoshie *et al.*, 2007).

2.8. Bacterial Profile

The microbial flora that develops in a pseudo pocket is predominantly anaerobic (Huang *et al.*, 2020). The microbial flora linked to pericoronitis is mixed and dominated by anaerobic bacteria, according to numerous microbiologic investigations using culture-dependent and culture-independent methods(Barrak *et al.*, 2020). *Streptococcus mutans, Prevotella intermedia,* and *Tannerella forsythia* are the common bacteria in the pericoronitis area(Ari Rajasuo *et al.*, 2012)

2.8.1. *Streptococcus Mutans*

Streptococcus mutans can be identified as the most important agent that cause dental caries among individuals (Mallya and Mallya, 2020). Its tendency to create a biofilm on tooth surfaces known as dental plaque, is one of its most essential pathogenicity features(Emeka *et al.*, 2020). The food debris that accumulates beneath the pericoronal area will lead to plaque formation and then caries lesion to the third molar, *Streptococci* are among the first bacteria to colonize oral surfaces and may make up 70% of the cultivable bacteria found in human dental plaque (Ebi *et al.*, 2001).

S. mutans are facultative anaerobes that are gram-positive, catalase-negative, cocci-shaped, and frequently discovered in the human oral cavity. It is associated with dental caries, also referred to as teeth decay, and is thought to be a typical component of oral flora. The previous study shows that all patients are affected by active dental infections rather than pericoronitis which produces a different bacterial biofilm (Fating *et al.*, 2014). *S. mutans* is reported to be the most cariogenic bacteria due to its acidic produce and dental adhesion characteristics (Aval *et al.*, 2022)

Polymerase chain reaction (PCR) detection of bacteria is more sensitive and specific than traditional culture methods (Kim, 2021).

2.8.2. *Prevotella Intermedia*

Prevotella intermedia, a gram-negative, anaerobic pathogenic bacteria, is commonly found in acute necrotizing ulcerative gingivitis and causes periodontal diseases such as gingivitis, periodontitis, and pericoronitis(Izidoro *et al.*, 2022). It is frequently kept separate from oral abscesses, which are primarily dominated by obligate anaerobes. People with necrotizing ulcerative stomatitis are more likely to have *P. intermedia*(Termeie, 2020). It is able to form biofilms on tooth surfaces and within periodontal pockets, which can protect it from the host immune system and antimicrobial agents. Several studies have shown that *Prevotella intermedia* is frequently detected in pericoronitis-associated dental abscesses. *Prevotella intermedia* was one of the dominant species in pericoronitis and it can produce several virulence factors that contribute to its pathogenicity, including proteases, lipases, and endotoxins (Hockham, 2001). These virulence factors can lead to tissue destruction, bone loss, and inflammation in the periodontal tissues, which are characteristic features of pericoronitis (Yousefi *et al.*, 2020).

Overall, *Prevotella intermedia* is considered to be an important bacterium in the development and progression of pericoronitis. Its ability to produce enzymes that can break down collagen, which is a major component of the periodontal ligament that surrounds the tooth makes it a challenging bacterium to treat in this context.

2.8.3. *Tannerella Forsythia*

Tannerella forsythia is a Gram-negative anaerobic bacterium that is commonly found in the oral cavity of humans. T. forsythia is a pathogenic bacterium that is associated with chronic periodontitis, a common inflammatory disease that affects the gums and supporting tissues of the teeth (Chinthamani *et al.*, 2021). It is a member of the *Bacteroidetes phylum* and was previously known as *Bacteroides forsythias* (Cionca *et al*., 2010).

A study by Kim et al. (2019) found that *Tannerella forsythia* was one of the most commonly detected bacteria in pericoronitis samples from patients with mandibular third molars. *Tannerella forsythia* as one of the dominant species in pericoronitis-associated dental abscesses. It is able to form biofilms on tooth surfaces and within periodontal pockets, which can protect it from the host immune system and antimicrobial agents (Hockham, 2001).

Tannerella forsythia has been shown to have several virulence factors that contribute to its pathogenicity, including the production of enzymes that can degrade host tissues, such as collagen and elastin. These enzymes can lead to destruction of the periodontal tissues and bone loss, which are characteristic features of pericoronitis.

Overall, *Tannerella forsythia* is considered a key player in the development and progression of pericoronitis. Its ability to degrade host tissues make it a challenging bacterium to study in this context.

2.9. Enzyme-linked immunosorbent assay (ELISA)

It's a technique widely used in biomedical research for the quantitative and qualitative identification of specific antigens or antibodies in a given sample; it is also widely used as a diagnostic tool in medicine and quality control for many industries(Anwar *et al.*, 2016). ELISA is a biochemical test that is used to detect the presence and quantity of specific antigens such as proteins, hormones, peptides, and so on, or antibodies in samples, by using antibodies and enzyme-mediated color change to estimate the quantity of the target(Gan & Patel, 2013). The assay is based upon an antigen-antibody interaction and subsequent enzymatic action on a substrate yielding a soluble colored product (Crowther, 2009). Sandwich ELISA used in this study, this assay requires two types of antibodies the capture and detection antibodies, in addition, the target antigen must contain at less two antigenic locales; Each antibody is thus specific for one particular and non-overlapping antigen epitope(Vilím *et al.*, 2003). ELISA plate is coated with a capture antibody which acts to capture the antigen when added. Then detection antibody is added to bind with captured antigens so it is called a sandwich.

2.10. Molecular detection of bacteria

Real-time polymerase chain reaction used is a nucleic acid amplification test that provides a computerized diagnostic technique with increased sensitivity and specificity for the identification of bacteria (Dreier *et al.*, 2007). Fluorescent probes or fluorescent DNA-binding dyes and real-time PCR instruments that measure fluorescence while performing the thermal cycling required for the PCR reaction are

used to quantify the amplified product (An *et al.*, 2020). q-PCR enables the measurement of the product as the process is happening in real-time, in contrast to conventional PCR, which quantification, if done, is based on a "end-point" study of the amount of the amplicon.

A "closed-tube" method is used to quantify *Streptococcus mutans*, *Prevotella intermedia*, and *Tannerella forsythia* in real-time PCR enables measurement and analysis during amplification (Nolan *et al.*, 2006) by using additional spotting systems that utilize melting curve analysis and/or fluorescent identification systems. The real time PCR dynamic nature that can detect, quantify, other than genotyping gave this test a superiority in comparison with other techniques. The fluorescent molecules are screened using an optical thermocycler by activation and measurement of the fluorescent radiation. Although fluorophores can be designed in a variety of ways, such as by direct bound to double-stranded DNA or linked to an oligonucleotide to create a labeled primer or probe, the most frequent requirement is that there needs to be a change in fluorescence during the PCR process that would allow real-time monitoring of the product (Langlois *et al.*, 2021).

The original template content typically defines the number of cycles required in an optimized real-time PCR process before the fluorescence increases. When amounts are below the detection limits; the early exponential increase cannot be seen. This is followed by a period of development, and then a plateau. The divided cycle number is oppositely correlated to the log of the initial template concentration and fits with the point of greatest acceleration of this development progress (Paik *et al.*, 2022).

Melting curve analysis after amplification of these bacteria using in combination with real-time PCR as a probable identity of the target sample that amplified. SYBR Green dye is included in the real-time PCR reaction, and as the annealed products

melt at a steady rate and the double strands dissociate, the decline in fluorescence will be watched (Loghavi, 2016).

3.Materials and Methods

The participants of this study were (80) subjects they were divided into two categories:

1. Control group (C): included (30) subjects who were healthy individuals 17 males and 13 females.
2. Patient group (P): included (50) subjects with pericoronitis 17 males and 33 females.

3.1 Clinical investigations

Saliva and swab collection for clinical investigation with the help of a sterilized mirror, probe, cheek retractor, tweezer, and cotton roll. The sample was collected from subjects with acute or subacute pericoronitis. When the peri coronal pocket was probed, localized mucous redness, edema, discomfort, trismus, and pus were noted as symptoms of the disease. Regional lymphadenopathy and fever have also been observed in severe situations. The radiolucency in the x-ray of bone distally to the crown of the mandibular third tooth was the radiographic signature of pericoronitis.

3.2. Ethical Approval

Before any samples are collected, all individuals are properly informed, and informed consent is acquired to conduct the research and publish the results.

3.3. Inclusion requirements include:

- Patients with acute or subacute pericoronitis.
- Age above 16 years.
- Both gender.
- No antibiotic taken during the last 30 days.
- Clinically tested partial eruption third molar was included.
- No smoker.

3.4. Exclusion requirements include:

- All individuals under 16 years old.
- Patients with a systemic illness and autoimmune disease.
- Pregnant or lactating woman.
- Smoker.
- Person takes the systemic antibiotic or anti-inflammatory therapy.

3.5. Supplies, Tools, and Equipment

3.5.1. A summary of the materials, tools, and instrument was provided in Table (3-1).

Table (3-1): Materials, Equipment, and Instruments.

No.	Instrument	Company	Country
1	Diagnostic instrument (Disposable mirrors, probes, and tweezers)	Siladent	China
2	Absorbent Paper points, Cotton roll	Diadent	China
3	Lab Test Tubes	Kang Jian	China
4	Pen marker	Wanniansheng	China
5	Coolbox with an ice bag	Colemon	China
6	Centrifuge	Biotek	Germany
7	Adjustable micropipette (different size)	Dragon	China
8	Disposable micropipette tips	Dragon	China
9	An Eppendorf tubes 2.5 ml	Dragon	China

10	water bath	Memmert	Germany
11	Incubator	Memmert	Germany
12	Vortex	Quality Lab System	England
13	microcentrifuge tube 1.5ml	Haimen United Laboratory Co.	china
14	Thermal Cycler	Rotor-Gene Q (Qiagen)	Germany
15	Racks fits 1.5, 2.0, and 0.2 milliliter tubes		China
16	PCR tubes		China
17	Biohazard waste container		China
18	Laminar safety box		China
19	Disposable medical non-sterile powder-free gloves	Falcon	Malaysia
20	Disposable Face mask 3PLY	Shanghai hau En industrial co.	China
21	Autoclave	Hirayama	Japan
22	Eppendorf centrifuge	Hettich	Germany

3.5.2. Chemical Materials

The chemical materials which are utilized in the present study are listed in **Table (3-2).**

Table (3-2): Chemical Materials

No.	Biological and Chemical Materials	Manufacturer
1	Phosphate buffer tablet	Himedia (India)
2	Absolute Ethanol	BDH (England)
3	Nuclease Free Water	Bioneer (Korea)
4	Pcr master mix	Cyntol Russia
5	MgCl2	Cyntol Russia
6	2xPerfectStartIM Green qPCR SuperMix	Cyntol Russia

3.6. Design of the Study:

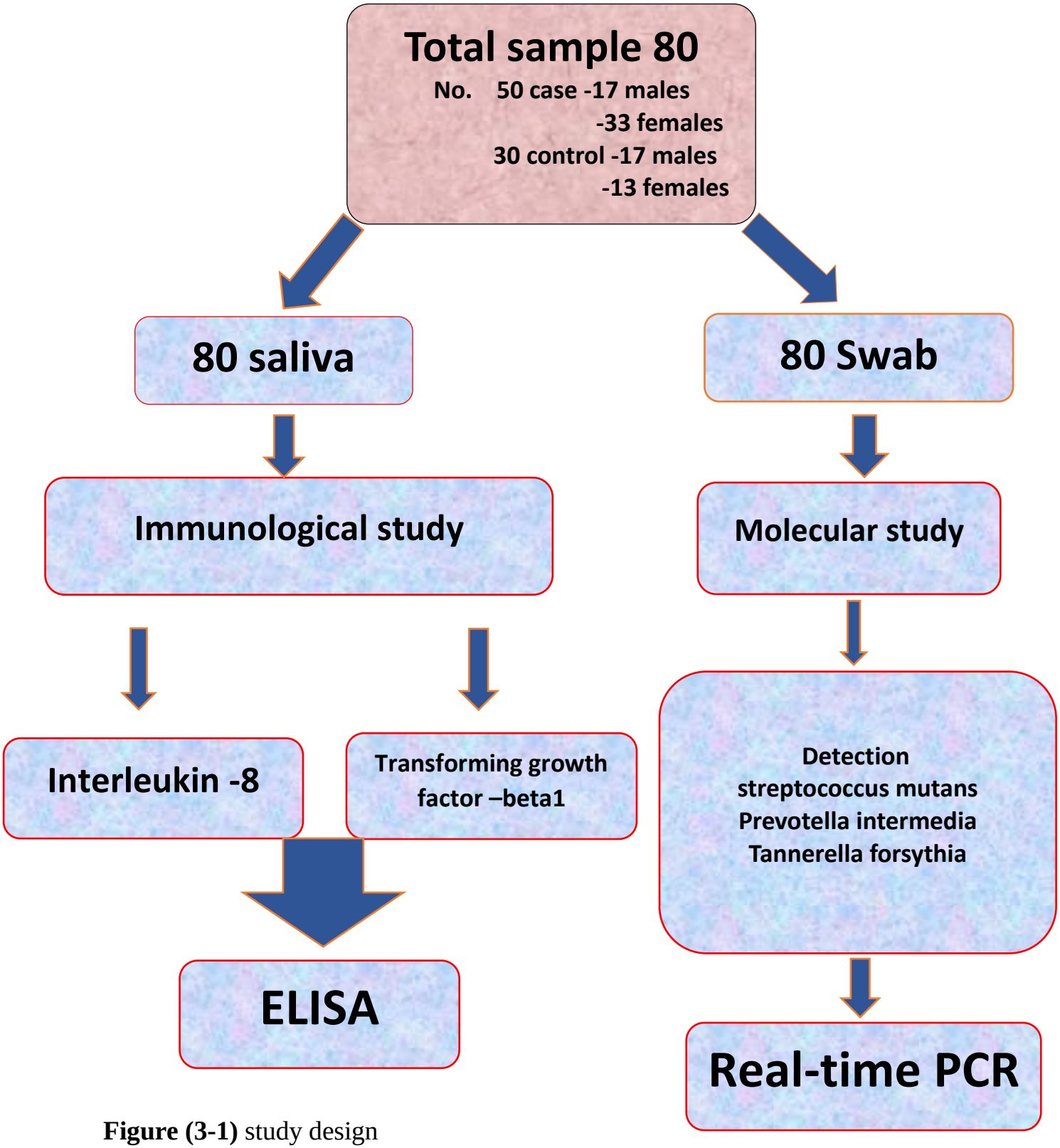


Figure (3-1) study design

3.7. Sample collection:

3.7.1. Saliva Sampling

Saliva samples are collected properly in a contamination-free procedure for each patient. In addition to the bacteriological sample, the mouth is rinsed with distilled water (10ml) for 30-60 seconds to ensure the removal of any debris. The patient is instructed not to swallow or move his tongue or lips during the time of the saliva collection, according to Prieto's recommended method. The patient is instructed to spit the accumulated saliva into the receiving vessel after the saliva had been allowed to sit in the mouth for two minutes(Sreebny, 1992). Unstimulated saliva samples are centrifuged in 2 ml portions for 15 minutes at 4000 rpm. The clear supernatant is then removed with a micropipette, placed in plain containers, and kept in a deep freezer at (-20 C) to make the final immunological examination study by the enzyme-linked immunosorbent assay (ELISA) test.

3.7.2. Swab sampling

Sample collection is carried out by isolation the lower third molar and preventing saliva contamination by cotton roll and saliva aspiration. Gently dry the area by air syringe then a sterile endodontic 3 paper point (size 20) is placed at the deepest points of the pseudo pockets, under the soft tissue of peri coronal area of the third molar until resistance felled and wait for 30 seconds. Then the paper point is pulled away and immediately put in an Eppendorf tube containing (phosphate buffer solution) and labeled. The label contains a patient's name, gender, number of a tooth from which sample is taken, type of sample, and date of collection. The sterile paper points are placed in the distal region of the mandibular third molar and held for 30 seconds to collect samples from individuals in the control group. Then the tube is stored at (-20C) in a deep freeze until it is used for real-time PCR technique as shown in figure (3-2)

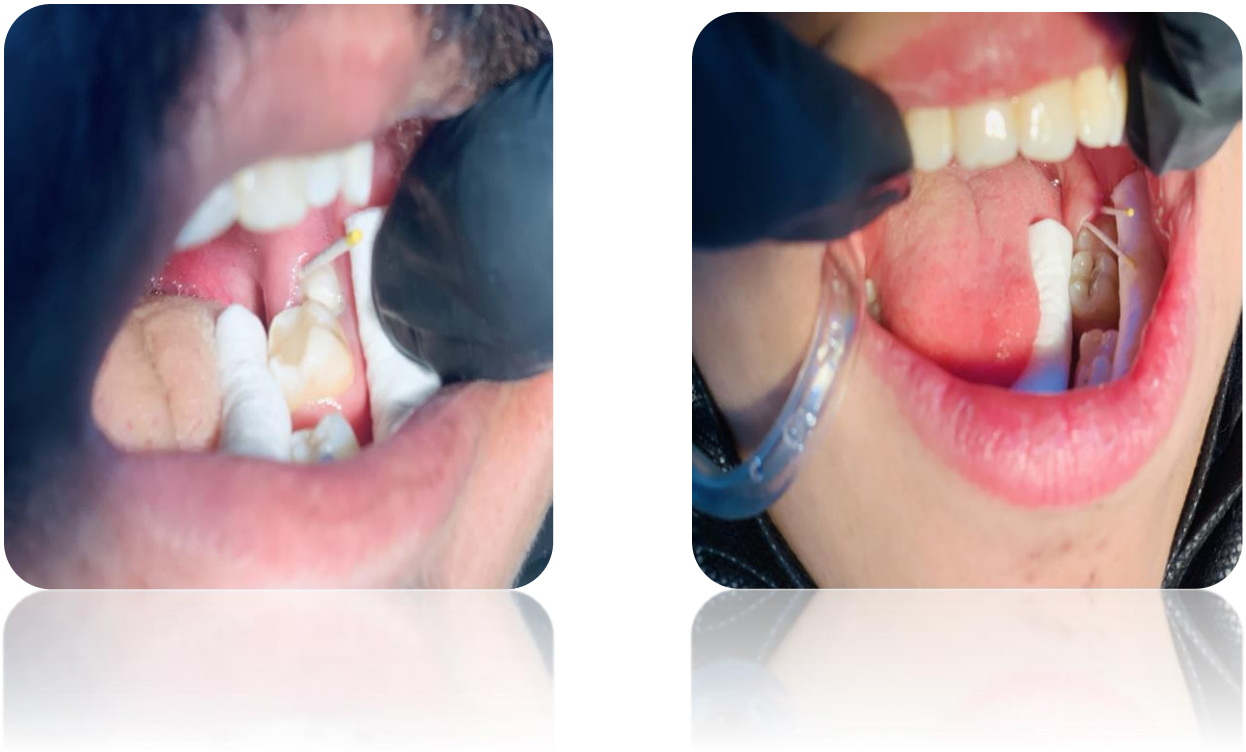


Figure (3-2) bacterial swab collection

3.8. Preparation of Solutions used in the present study:

Phosphate Buffer Saline (PBS) One tablet of ready-to-use phosphate buffer saline (PBS) is dissolved in 100 ml of D.W.

3.9. Molecular detection of bacteria:

3.9.1. DNA extraction

The DNA extraction using phenol/chloroform manual method has been improved by the DNA extraction strategy described in this research (Cheng AND Jiang, 2006).

• Eppendorf tube that has bacteria will Centrifuge at 10,000 rpm for 2 minutes.

• Discard the supernatant and wash the pellet twice with a suspension.

• Suspend the pellet in 200 µl of TE buffer, which has a pH of 8.0, and is composed of 10 mM Tris/HCl, and 1 mM EDTA.

• Add 100 µl saturated phenol with Tris-EDTA (TE) buffer.

• Vortex for 60 seconds.

• Centrifuge the material at 10,000 rpm for 5 minutes to separate the aqueous phase and organic phase from them.

• Take 160 µl from the aqueous layer and transfer them to a new tube.

• Add 40 µl of TE buffer and 100 µl of chloroform.

• Centrifuge at 10,000 rpm for 5 minutes.

• Take the supernatant and repeat the addition of chloroform and centrifugation until the white layer disappears (The chloroform will be in the lower organic layer, while the DNA will be in the upper aqueous layer separated by a white layer of proteins).

• Discard the white layer of proteins by repeating the addition of chloroform.

• Take 160 µl of the supernatant to a new tube.

• Add 40 µl of TE buffer and 5 µl of RNase to digested RNA.

• Incubate for 15 minutes at 37 ℃.

• Add 100 µl of chloroform.

• Vortex and centrifuge at 10,000 rpm for 5 minutes.

• Take 150 µl of the supernatant and transfer it to a new tube, which represents the pure DNA.

3.9.2. Bacterial detection by Real-time PCR

The detection was carried out by PCR Rotorgene Q (Qi agene, Germany) employing the primer and the real-time PCR reaction ingredient as listed in table (3-3).

Table (3-3) Volumes of solution for the real-time PCR protocol reaction

Component	**Total volume 50 µl**
2x Master mix Syper Green (2.5X)	20 µl
DNA (10-20 ng/ml)	4 µl
F - primer (10pmol/µl)	1 µl
R- primer (10pmol/µl)	1 µl
MgCl2 (25 mM)	1 µl
Nuclease free water (N.F.W)	23 µl

1. To spin down the contents and get clear of any air bubbles, the tube where all the components are collected by centrifuge.
2. The program is run after the tube is placed inside the PCR well.
3. The program conditions for the real-time PCR are specified for research as shown in table (3-5)

3.9.3. Real-time PCR conditions

The suitable PCR cycling program conditions are installed, and the PCR tubes are put on the PCR machine as in Table (3-4)

Table (3-4) Thermocycler Conditions for PCR

Steps	Temperature	Time	N. of cycle
"Initial denaturation"	95 C°	5 min.	1
"Denaturation"	95 C°	30 sec.	40
"Annealing"	55 C°	30 sec.	
"Extension"	72 C°	30 sec.	
"Final extension"	72 C° Melt 60-95 ºC for each step,5 sec.	5 min	1

3.9.4. Primers

The following primers are used in this study to identify the target genes in bacteria as listed in Table (3-5)

Table (3-5) primer for bacterial detection

Bacteria	**Primer**	**reference**	**Product size**
Streptococcus mutants	F AGCCATGCGCAATCAACAGGTT R CGCAACGCGAACATCTTGATCAG	(Vieira *et al.*, 2011)	415 bp
Prevotella intermedia	F CGTATCCAACCTTCCCTCC R ATTAGCCGGTCCTTCGAAG	(Moraes *et al.*, 2015)	389 bp
Tannerella forsythia	F GCGTATGTAACCTGCCCGCA R TGCTTCAGTGTCAGTTATACCT	(Sakamoto *et al.*, 2001)	360 bp

3.9.5. Data Analysis for the studied *bacteria* by real-time PCR

3.9.5.A. Detection of *Streptococcus mutans, Prevotella intermedia and Tannerella forsythia* by real time PCR

-Amplification curve: By determining the threshold cycle number (CT value) that displayed the positive amplification (red line) and negative (blue line) as shown in appendix.

-Melting curve: The selectivity and specificity for all analysis are checked by melting curve analysis.

3.10. Immunological study

3.10.1. Estimation of Immunological Cytokines (ELISA elabscience Kits-interleukin-8 and transforming growth factor beta-1)

Table: (3-6) immunological Kits.

No.	Name of Kit	Company/ Origin
2	Human Interleukin 8 ELISA Kit	Elabscience \ China
1	Human TGF-B1 ELISA Kit	Elabscience \ China

3.10.1.A. Test Principle

ELISA package is getting a system called Sandwich-ELISA, the principle of the test as follows explained.

According to object protein, the micro ELISA plate of Kit has only been pre-coated with an antibody specific to Human IL-8 and TGF-B1. The Micro-plate wells of ELISA were packed with standards or samples and linked with specific antibody, then a biotinylated detection antibody selected for Human IL-8, and TGF-B1 and HRP Peroxidase (HRP) conjugate added successively to every well of the microplate. After that, they incubated for one hour at least in the incubation or overnight in the refrigerator to get the reaction between the antigen and two types of antibodies, free components which did not link to the antigen-antibody complex are washed. And then, the substrate solution is put to every well of the micro plate. Only wells which have Human IL-8 or TGF-B1 detection antibody, which reacts with HRP conjugate, will be visible in different degrees of blue color based on reaction intensity. The reaction is finished by the addition of strong acids or strong base, and the color has become yellow. 0ptical density (OD) is recorded with an ELISA reader at a wavelength of 450 nm ± 2 nm. Concentration rate of Human IL-8 or TGF-B1 is directly proportional to the optical density, as the concentration increases as the OD increases. So the results can be calculated through the straight line equation and represented on the curve point to point through a unique software program linked to the computer (Gan & Patel, 2013).

Standard working solution, Centrifuge the standard at 10,000 rpm for 1 minute. Add 1.0 mL of reference Standard and sample diluent, let stand 10 min, then gently invert. Pipette it once it's dissolved. The reconstituted solution is 1000 pg/mL. (Alternatively add 1 mL of Reference Standard & Sample Diluent, let rest for 1-2 minutes, and thoroughly mix with a vortex meter set to low speed. Bubbles

formed during vortexing might be eliminated by centrifuging at a low speed). Occasionally serially dilute. 1000, 500, 250, 125, 62.5, 31.25, 15.63, 0 pg/mL. Fill 7 tubes with 500 mL of reference standard and sample diluent. Mix 500uL of the 1000 pg/mL working solution into the first tube. Pipette 500 mL of the first tube's solution into the second. (3-3) is a reference figure.

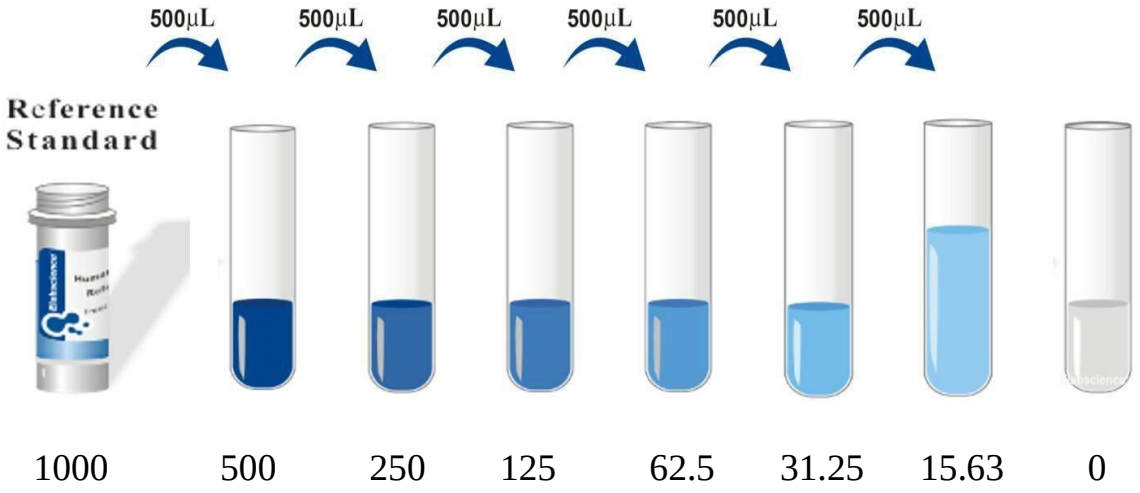


Figure (3-3): Dilution method reference standard (Elbscince)

3.10.1.B. Assay Procedure

The standards, samples, and reagents are prepared as instructed:

1. 100µL of standards and samples were added to every well. And then incubate at 37 °C for 90 minutes.
2. Immediately after removing of liquid, add 100 µL of the biotinylated detection antibody, then seal with plate sealer, lightly tap, and incubate at 37°C for 1 hour at least.
3. After being (aspirated and washed three times), the unbounded biotinylated antibody is taken away.

4. 100 µl working HRP conjugate solution was added, then incubate at 37 °C for 30 minutes. Once liquid has been eliminated, wash the plate five times.

5. Add 90 µl substrate reagent and incubate at 37°C until appearing of blue from (5-15) min.

6. When the stop solution 50 µl was added to the liquid, the enzyme- substrate reaction is finished and the color changed from blue to yellow.

7. Finally, read the plate at 450 nm immediately by the ELISA reader and the calculation of the results are automatically calculated according to the standard curve.

3.10.2. Calculate of results

A wavelength of 450 nm is used to spectrophotometrically determine the optical density (OD). The OD number correlates with the levels of human IL-8 and TGF-beta 1. The concentration is calculated by comparing the OD of the samples to the standard curve. Concentration of sample is equal to OD sample/OD standard plus Concentration of standard.

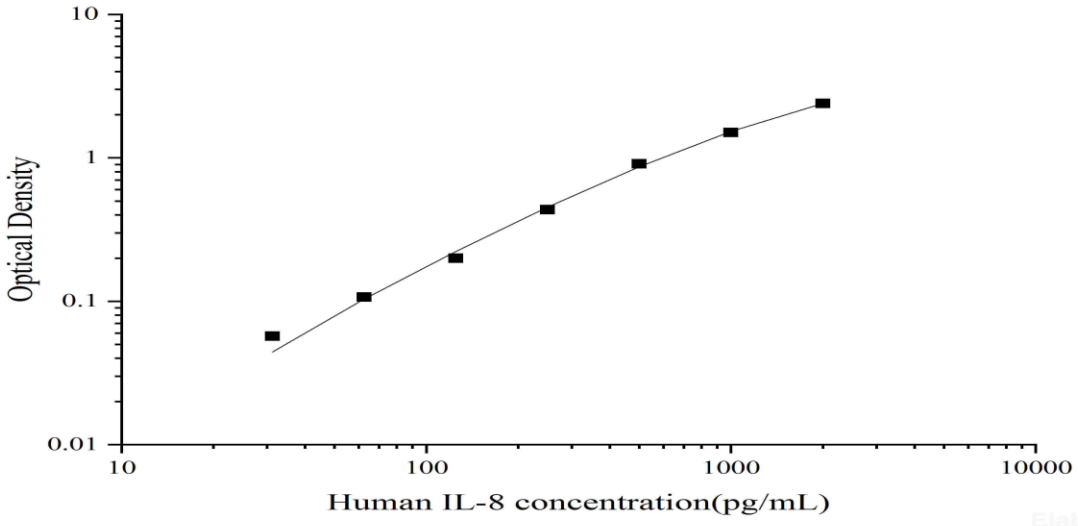


Figure (3-4) IL-8 standard curve.

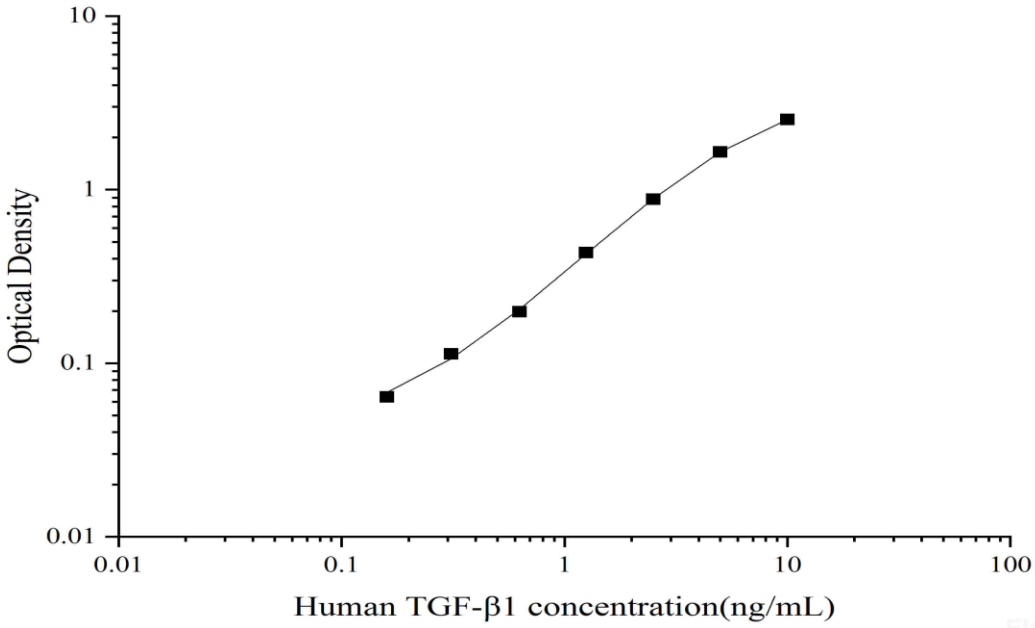


Figure (3-4) TGF-b1 standard curve.

3.10.3. Statistical analysis.

For descriptive statistics the statistical software for the social sciences edition 23.0 (SPSS) program is used. Continuous variables are shown using means and standard deviations. To compare the p-values of groups, a chi-square calculator for independent samples is used. P-values below or equivalent to 0.05 defined as statistically significant, while P-values above 0.05 are defined insignificant.

4. Results

4.1. Demographic characteristics

The study includes 80 subjects, 50 of whom have pericoronitis which includes 17 males and 33 females with 30 control group which includes 17 males and 13 females at different times. The age of both groups range between (16-36) subdivided into two groups ≤ 26 years which includes 36 of the patient group and 22 of the control group and ≥ 27 years which includes 14 of the patient group and 8 of the control group as shown in Figures (4-1,4- 2, 4-3)

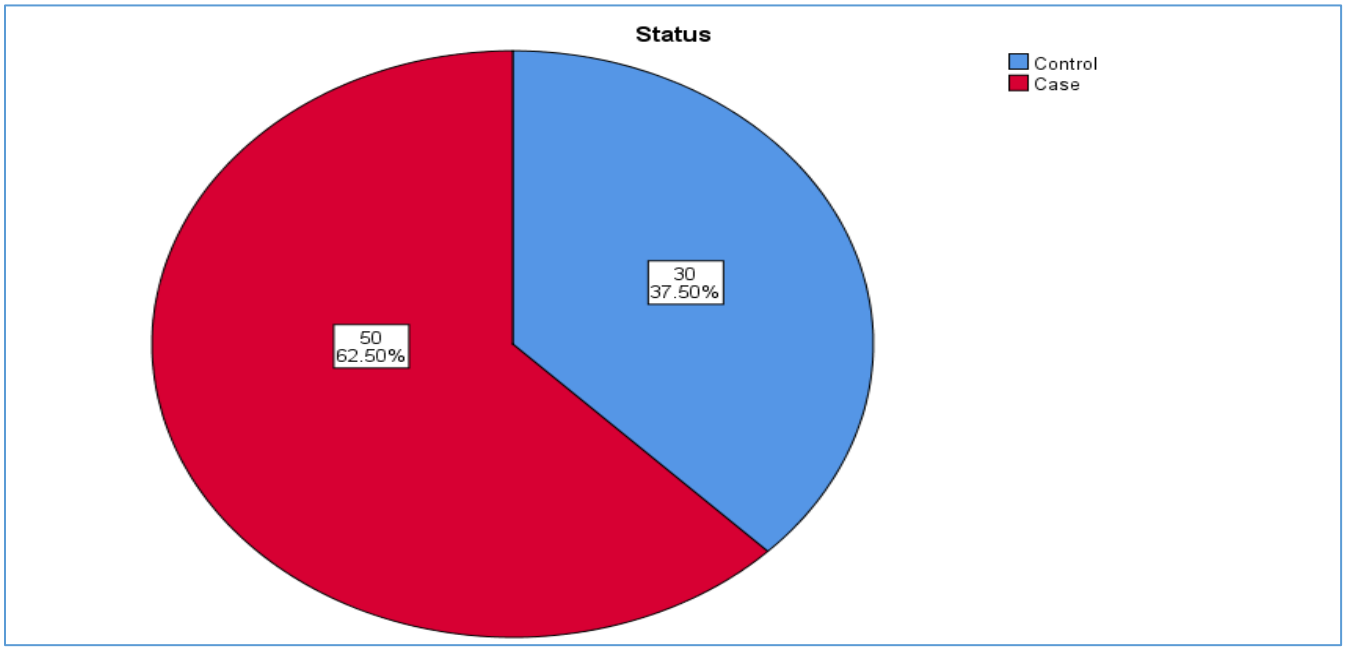


Figure (4-1) Distribution status of samples

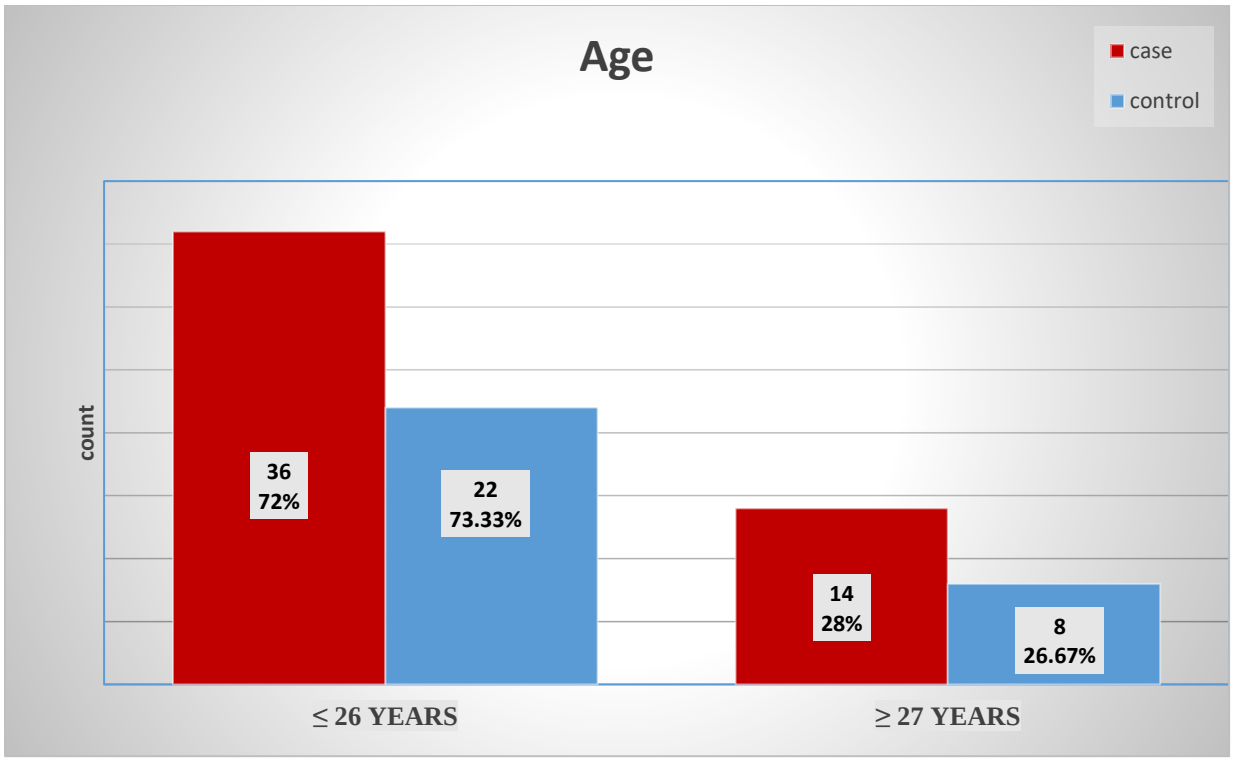


Figure (4-2): Age distribution of patients.

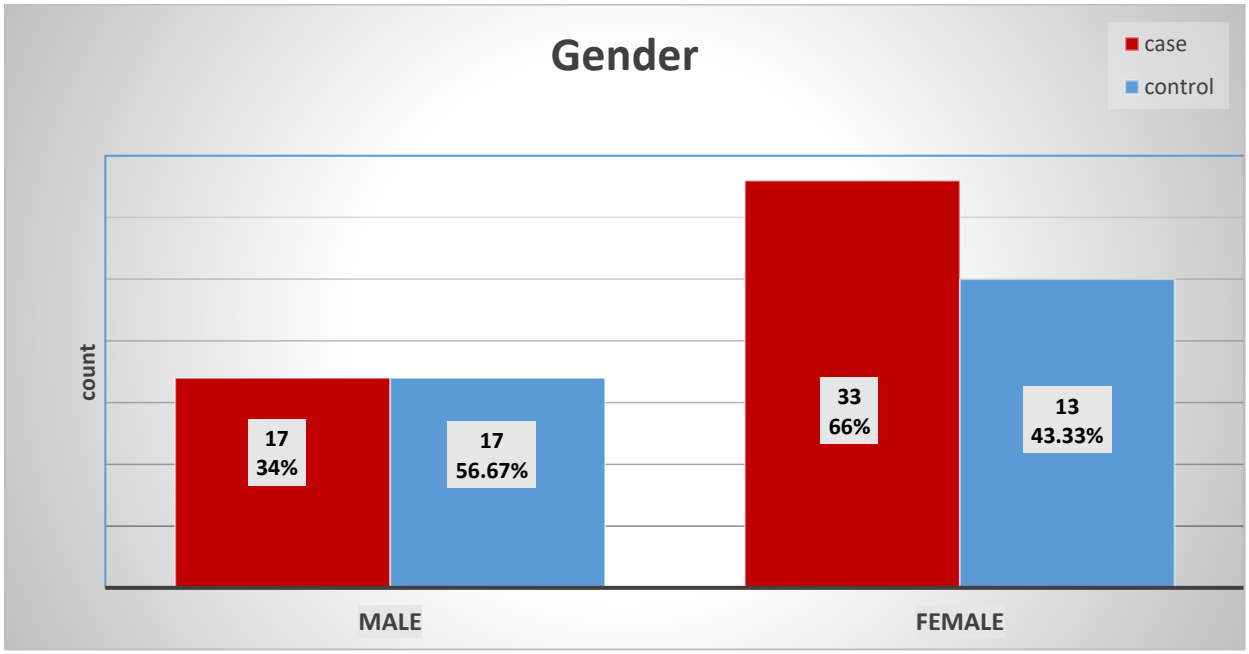


Figure (4-3): Gender distribution of patients.

4.2. Bacteriology study

4.2.1. Detection of *Streptococcus mutans* by RT-PCR technique:

In this study, 80 samples were detected for the presence of *streptococcus mutans* by RT-PCR technique. The results showed that the presence of *S. mutans* where positive in 32 (64%) cases of the patient group, while its presence was negative in 18 (36%) cases of the same group, while in control groups the presence of *S. mutans* was positive in 5 (16.67%) cases and negative in 25 (83.33%) cases of this group, as shown in figure (4-4).

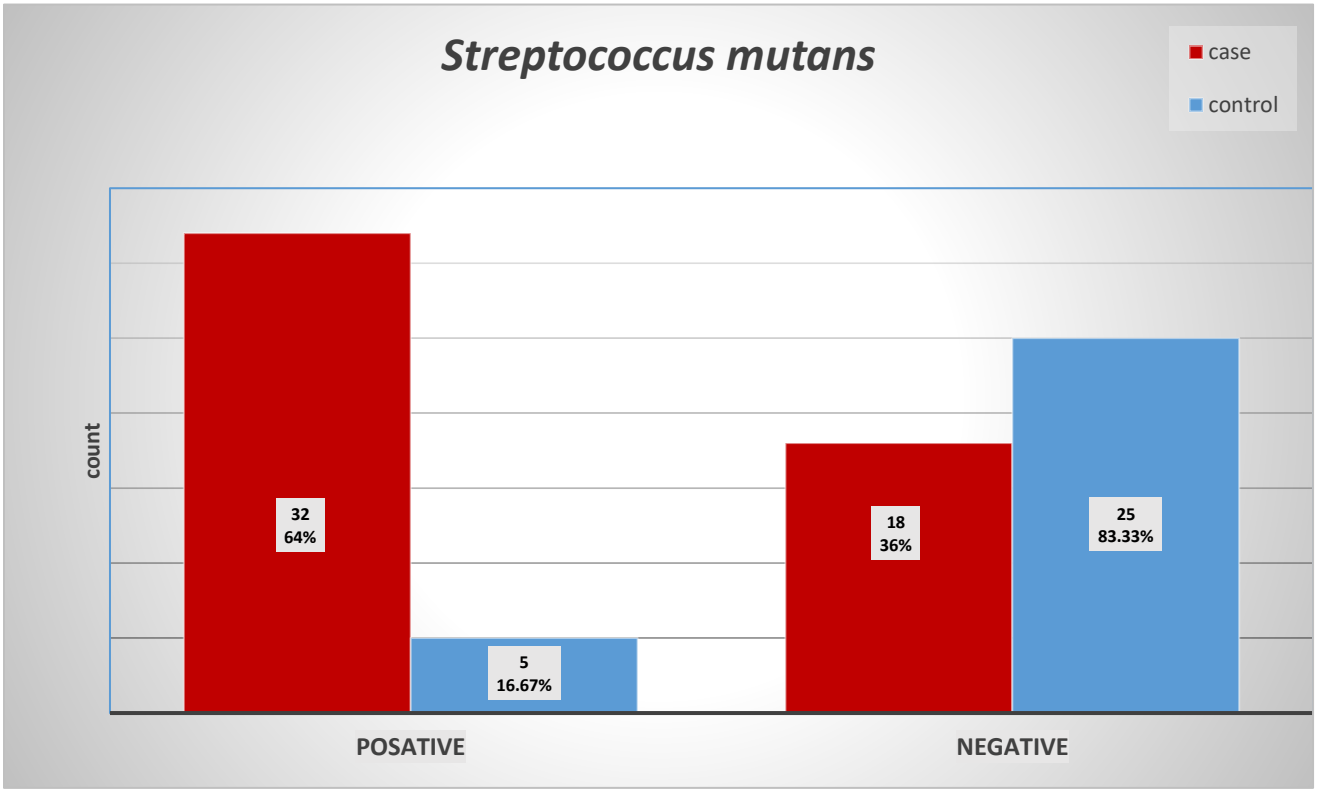


Figure (4-4) Distribution of *streptococcus mutants* among patients and control group.

4.2.2. Detection of *Prevotella intermedia* by RT-PCR technique:

In this study, 80 samples were detected for the presence of *Prevotella intermedia* by RT-PCR technique. The results showed that there was a presence of bacteria where positive in 29 (58%) cases of patient group and negative in 21 (42%)cases of the same group while in control group the presence of *P. intermedia* was positive in 1(3.33%) cases and negative in 29 (96.67%) of the same group as shown in figure (4-5).

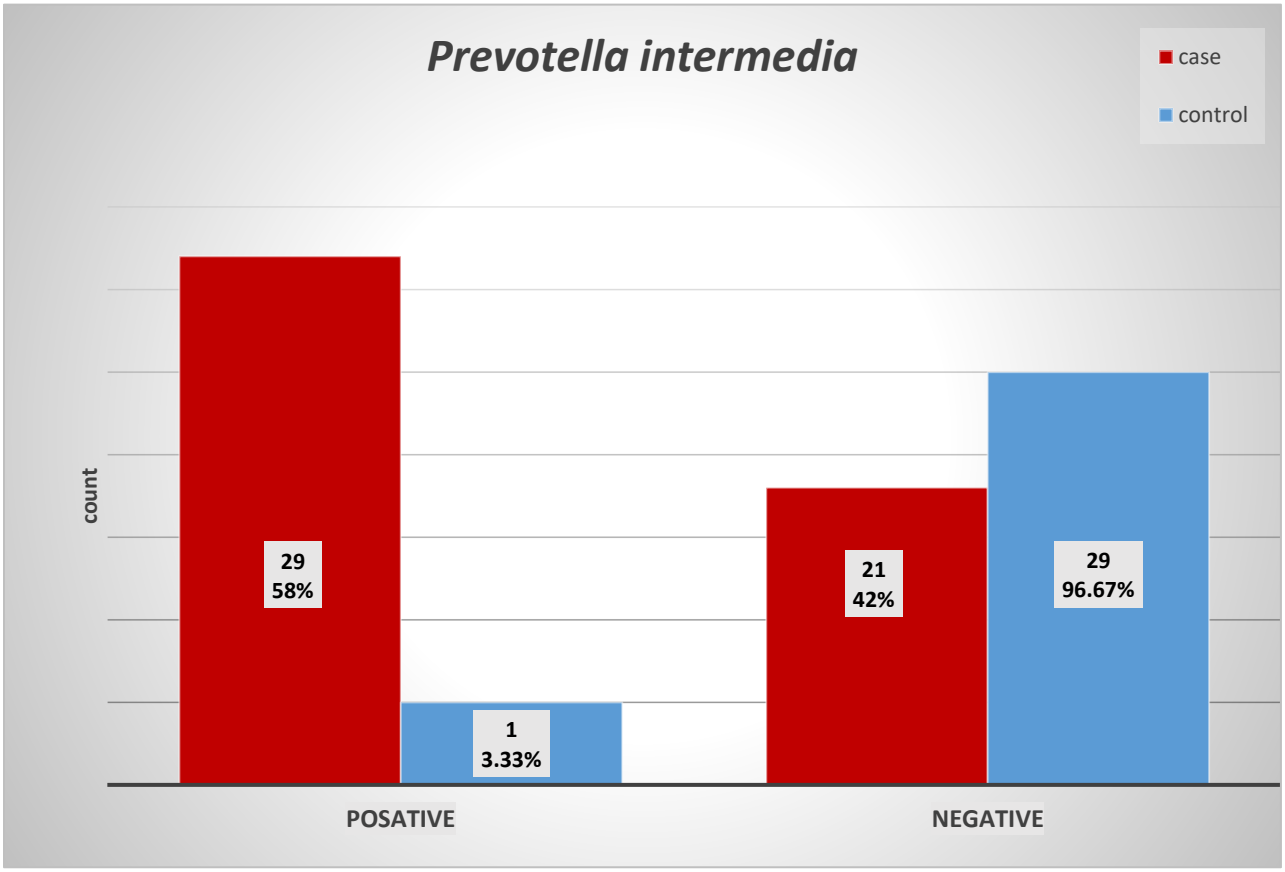


Figure (4-5) Distribution of *Prevotella intermedia* among patients and control group.

4.2.3. Detection of *Tannerella forsythia* by RT-PCR technique:

In this study, 80 samples were detected for the presence of *Tannerella forsythia* by RT-PCR technique. The results showed that there was a presence of bacteria was positive in 18 (36%) cases of patient group and negative in 32 (64%) cases of the same group, while in control group found that the bacteria were negative in all samples as shown in figure (4-6).

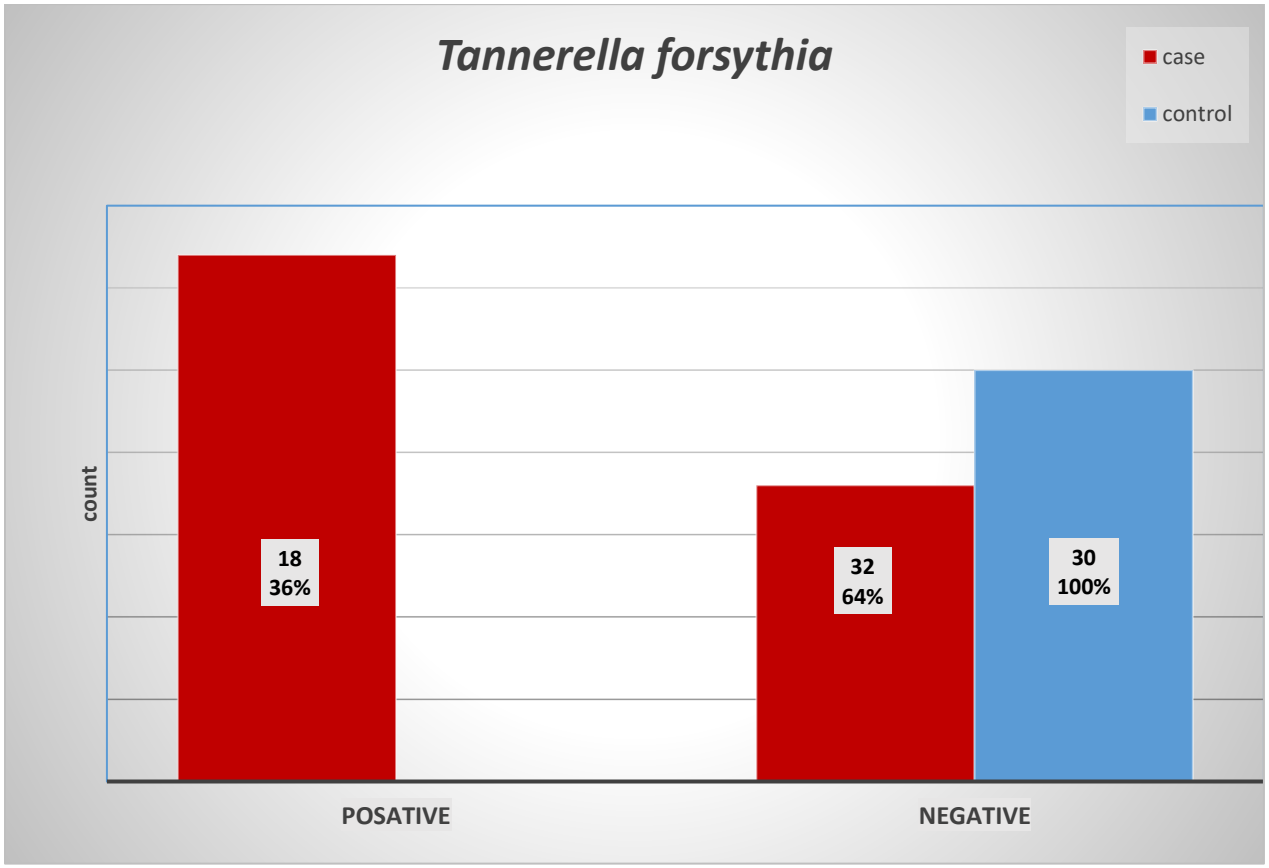


Figure (4-6) Distribution of *Tannerella forsythia* among patients and control group.

4.3. Immunological study

4.3.1. Determination of interleukin-8 (IL-8) concentration in saliva among pericoronitis patients and control groups

The result shows that the mean±S.D concentration of IL-8 in saliva for patient group (was 145.66±28.1) while for control group was (53.16±13.03) which showed highly significant differences, as shown in table (4-5) and figure (4-7) showed that the patient's groups had high IL-8 concentration compared with the control group.

Table (4-5) Determine IL-8 concentration among patients and control group.

Status		N	Mean	Std. Deviation	P-value
IL-8	Control	30 (37.5%)	53.1600	± 13.03104	
	Case	50 (62.5%)	145.6646	± 28.10318	*0.000

* Significant level >0.05

*Non-Significant level ≤ 0.05

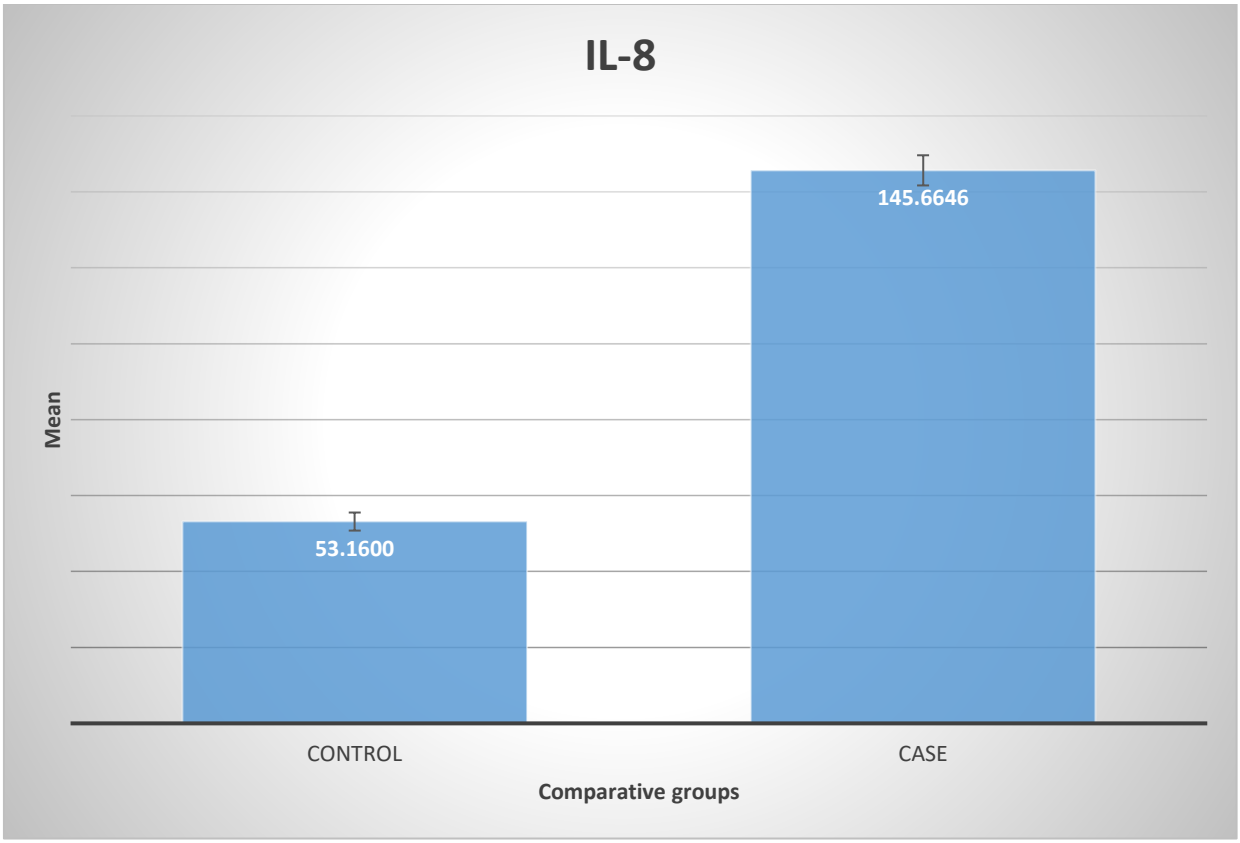


Figure (4-7) Concentration of IL-8 among pericoronitis patients and control Group

4.3.2. Determination of transforming growth factor- beta 1 (TGF-B1) concentration in saliva among pericoronitis patients and control groups:

The result shows that the mean±S.D concentration of TGF-B1 in saliva for patient group was (271.55±36.23) while control group was (119.33±27.67) which are highly significant differences, as shown in table (4-6) and figure (4-8) shown that the patient's groups had high TGF-B1 concentration compared with the control groups.

Table (4-6) Determine TGF-B1 concentration among patients and control groups.

Status		N	Mean	Std. Deviation	P-value
TGF-B1	Control	30 (37.5%)	119.3387	± 27.67444	
	Case	50 (62.5%)	271.5564	± 36.23487	*0.000

* Significant level >0.05

*Non-Significant level ≤ 0.05

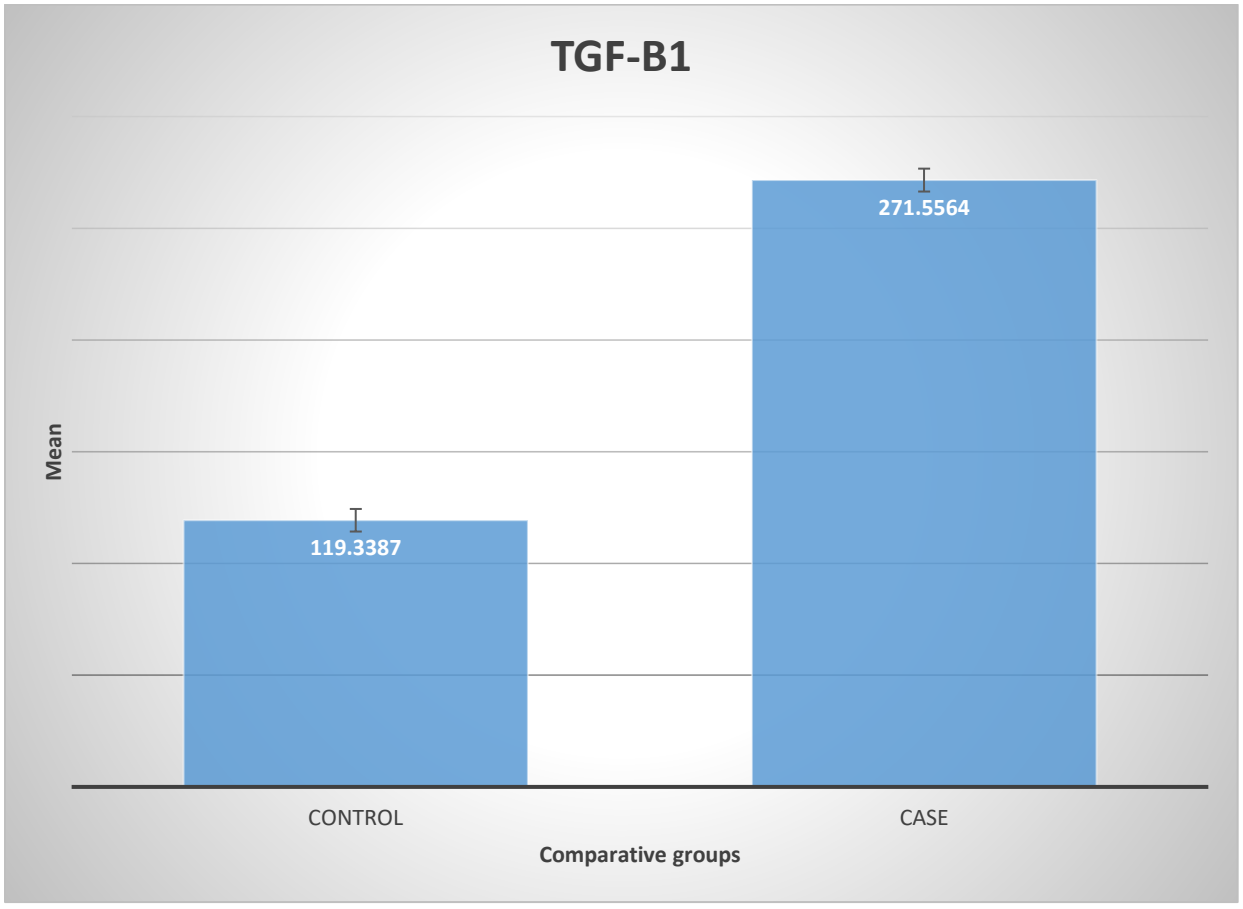


Figure (4-8) Concentration of TGF-B1 among pericoronitis patients and control group.

4.4. Correlation between study parameters:

Table (4-7): The Correlation between IL-8 and TGF-B1

Correlations		
		TGF-B1
IL-8	Correlation r	0.792**
	P-value	0.000
	N	50
**. Correlation is significant at the 0.01 level (2-tailed). Significant level >0.05		

This table showed the correlation between interleukin-8 and transforming growth factor-beta1 in pericoronitis cases is strongly positive with high significance (p-value 0.000). table (4-7) and figure (4-9).

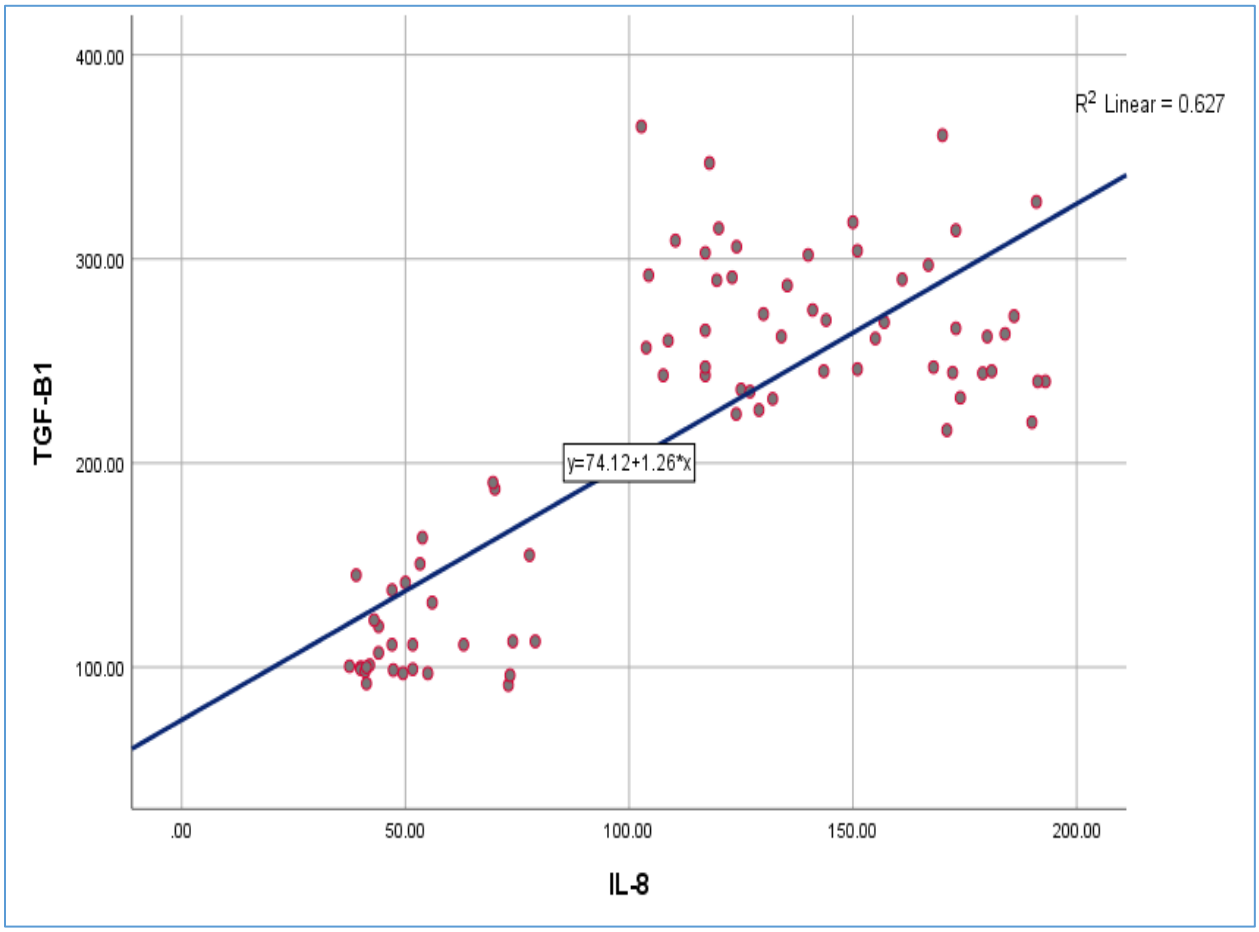


Figure (4-9) positive correlation between interleukin-8 and transforming growth factor-beta1

5-Discussion

Factors associated with the development of such an infection are poor oral hygiene, trauma from the opposing tooth on the soft tissues, smoking, and upper respiratory tract infection.

5-1 Distribution of pericoronitis infections according to gender and age among patients and control groups

The gender distribution of pericoronitis in this study revealed 33 cases (66%) in females and 17 cases (34%) in males which exhibited statistical significance. This observation may be attributed to a decrease in the immune system during hormonal changes and periods of stress, potentially resulting in alterations in the oral microbiome and an elevated risk of gum disease and other oral infections. Furthermore, females may have a higher likelihood of possessing smaller jaws or less space in their mouths for the eruption of wisdom teeth, thereby increasing the risk of impaction and pericoronitis. This result was agree with Bataineh who reported that pericoronitis cases were much seen in female patients (56.7%) than male patients (43.3%)(Bataineh & Al Qudah, 2003) and agreement with Yamalik study (Yamalık & Bozkaya, 2008). The present results disagree with several studies have reported the distribution of pericoronitis between the genders to be insignificant with a slight female predominance(P. Singh *et al.*, 2018) and disagree with result of Caymaz who found that there is no significant correlation of gender with pericoronitis (Caymaz & Buhara, 2021) .

According to the findings of the present study on pericoronitis infections, a total of 50 cases were identified. The analysis revealed a correlation between age

groups and the occurrence of pericoronitis. The age group between 16 and 26 years was found to be the most affected, with 36 cases (72%), while the age group above 27 years accounted for 14 cases (28%). This trend may be attributed to the incomplete eruption of the third molars through the gum line. Consequently, a flap of gum tissue forms, creating an environment where food particles and bacteria can become trapped, leading to the development of pericoronitis. This result are in agreement with Yamalik result who found that the incidence peak of pericoronitis that occur around lower third molar was seen between 19-23 years age group which is the period when mandibular third molars normally erupt (Yamalık & Bozkaya, 2008). The present results was in disagreement with the results of Caymaz who explained that there is no significant correlation between age with pericoronitis (Caymaz & Buhara, 2021).

5.2 Bacteriological study

In this study, (50) samples were collected from patients to detect the presence of *Streptococcus mutants*, *Prevotella intermedia,* and *Tannerella forsythia* by real time-PCR technique. Results revealed based on the amplification curves, where the red line indicated positive amplification above the cycle threshold (CT value), and the blue line represented negative result. The melting curve figures in the appendix were utilized to assess the selectivity and specificity of the study's analysis once the amplification cycles were completed. The peak of the red line in the melting curve represented the temperature at which the double-stranded DNA separated into single strands, causing a decrease in fluorescence due to dye dissociation.

5.2.1 Identification of *Streptococcus mutans* by real-time PCR:

Streptococcus mutants are considered one of the most important microorganisms in the etiology of dental diseases by acidic production (Priyadarshini & Gurunathan, 2020). This study set out with the aim of assessing the importance of real-time PCR in the detection of *S. mutans* about 32 bacteria in pericoronitis case and 5 in the control group was detected by molecular method positive results for PCR by using specific primer, 415 bp, this primer uses for *S. mutans* (Vieira *et al.*, 2011). The recorded results indicated that the gram-positive bacterium *S. mutans* was significantly predominant in 32 cases (64%) of the pericoronitis samples. In contrast, it was found in 5 cases (16.67%) of the control samples. The bacteria in question, *S. mutans*, were not detected in 18 cases (36%) of the pericoronitis samples. Similarly, they were not present in 25 cases (83.33%) of the control samples. This predominance may be due to its ability to cause dental caries which may progress to pulp infection, dental bad hygienic measurement with odontogenic infection, these microorganisms have several toxins that play crucial roles within host-pathogen interaction allowing the pathogen to colonize, proliferate and disseminate tissues (Espejo *et al.*, 2010).The present study were in agreement with Adnan *et al* .(2019) study who found *S.mutans* is most common bacteria in pericoronitis (Azemi *et al.*, 2019) .The study also agreed with Rajasuo and Wang who recorded *S. mutants* most common bacteria that cause dental infections (Ari Rajasuo *et al.*, 2012; Wang *et al.*, 2012).

5.2.2 Identification of *Prevotella intermedia and Tannerella forsythia* by real-time PCR:

Detection of these bacteria by real-time PCR technique in this study, a total of 80 samples were analyzed using specific primers for each bacterium. The results revealed that *Prevotella intermedia* was detected in 29 cases (58%) of the pericoronitis samples and in 1 case (3.33%) of the control samples which was significantly predominant. These bacteria were not present in 21 cases (42%) of the pericoronitis samples. Similarly, they were not present in 29 cases (96.67%) of the control samples as shown in figure (4-5). The present results agreed with Sixou *et al*.(2003) according to his study the most frequently detected microorganisms in pericoronitis microbiota were *P. intermedia (*Sixou *et al.,* 2003*)* and disagree with Laine *et al*.(2012) who found that the presence of *P. intermedia* is (35%) (Ari Rajasuo *et al.,* 2012).

A recent study by Sencimen *et al*. (2014) based on real-time PCR found statistically significant higher numbers of *Tannerella forsythia* detected in samples from pericoronitis patients(Sencimen *et al.,* 2014). The findings of this study agreed with a previous study, indicating the presence of *T. forsythia* in 18 cases (36%) of the pericoronitis samples. However, no presence of this bacterium was detected in the control samples make it highly significant. This bacterium was not present in 32 cases (64%) of the pericoronitis samples. Furthermore, none of the control samples showed the occurrence of this bacteria, accounting for 30 cases (100%) in the control samples as shown in figure (4-6). The findings of this study are consistent with previous research conducted by Sencimen *et al*. (2014) and A. Rajasuo *et al*. (2007). These studies reported that the presence of *T. forsythia* increases the risk of

developing pericoronitis by 8 times when compared to individuals without it. The present results are in disagreement with the findings of Reichhart *et al*. (2000) and Andric *et al*. (2017), who suggested that *T. forsythia* may be associated with the development of pericoronitis and reported its presence in a small number of their samples(Peltroche-Llacsahuanga *et al.*, 2000; Jakovljevic *et al.*, 2017).

5.3. immunological study

Although cytokines play essential roles in innate and adaptive immune responses, they have been found to have adverse impacts on human growth and bone metabolism(Lacey *et al.,* 2009). Recent studies have shown a link between periodontal disease and the production of pro-inflammatory cytokines similar to those seen in osteoclastic bone resorption(Koide *et al.*, 2010).

5.3.1. Interleukin-8 (IL-8) concentration among pericoronitis patients and control group:

IL-8 is a multifunctional cytokine that play a role in immune and inflammatory activities like recruitment and activation of neutrophils to sites of acute inflammation (Souto *et al.*, 2014). Thus, this study attempted to estimate and compare the level of IL8 in the saliva of a patient with pericoronitis and control groups. The result showed that the mean±S.D concentration of IL-8 in saliva for patients (was 145.66±28.1) while control groups (53.16±13.03) which have high significant statistical differences (p-value 000) between pericoronitis and control group, which revealed that the patient's group had high IL-8 concentration compared with the control group. Preshaw *et al*.(2016) reported on his study an increase in the concentration of salivary IL-8 in periodontal infection (Jaedicke *et al.*, 2016), and that in agreement with the present study and Punyani

study who found a significant correlation between IL-8 in saliva and pericoronitis disease (Punyani & Sathawane, 2013).

5.3.2. Transforming growth factor beta-1 (TGF-B1) concentration among pericoronitis patients and control group:

Transforming growth factor -beta1 is a multifunctional cytokine that regulates a change in cellular processes including growth, differentiation, apoptosis, angiogenesis, and immune reactions (Haque & Morris, 2017).The TGF-B1 is the most abundant isoform(S. K. Kim *et al.*, 2019). Macrophages that are frequently found in pericoronitis can produce a wide range of pro-inflammatory and anti-inflammatory mediators, as well as substances that enhance pro and anti-inflammatory substances. These substances act in the development and repair of these lesions TGF-β and cytokines that act at the beginning and regulation of inflammatory processes through the activation and differentiation of osteoclasts, activation, and proliferation of fibroblasts, production of collagen and neovascularization(Azeredo *et al.*, 2017). This study evaluates the level of TGF-beta 1 in the saliva of patients with pericoronitis and control groups which are highly significant statistics(p=000). This is the first study to measure TGF-B1 levels in pericoronitis. Pericoronitis affecting mandibular third molars in young adults may indicate a higher prevalence of underlying periodontal inflammatory disease compared to young adults who have retained third molars without pericoronitis as suggested by(Gelesko *et al.*, 2009) so This study aligns with the findings of Khalaf *et al.* (2014), who reported that patients with periodontitis exhibit altered immune responses and elevated levels of TGF-B1. In Khalaf et al.'s study, among the various inflammatory mediators analyzed, TGF-β1 consistently showed increased levels in the patient group.

5.3.3. The correlation of study parameters in pericoronitis.

The correlation of immune parameters was visible in pericoronitis patients through the strong correlation among them, as medical statistics showed in the table (4-7) a Significant link between interleukin-8 and transforming growth factor-beta 1 (p=000).

Proinflammatory cytokines such as IL-8, play an important role in attracting neutrophils to the infectious site to remove contaminating substances, whereas TGF-β1 acts to convert monocytes to macrophages, which play an important role in augmenting the inflammatory response and tissue debridement. Macrophages initiate the development of granulation tissue and release a variety of proinflammatory cytokines, including IL-8, and TGF-B1 (Haekkinen *et al.*, 2000; Barrientos *et al.*, 2008). The present study has an agreement with Fan's study which demonstrated that TGF-β1 and IL-8 significantly increased in periodontitis(Fan *et al.*, 2019) and also in agreement with Kim's study in the elevation of IL-8 concentration in pericoronitis disease (A. R. Kim *et al.*, 2017).

6. Conclusions and Recommendations

6.1 Conclusions

1. The present study investigates that the most frequently detected bacteria in pericoronitis cases were *Streptococcus mutans* (64%), *Prevotella intermedia* (58%), and *Tannerella forsythia* (36%) using real-time PCR.

2. There is a significant gender difference, however, females tend to be predominant over males regarding study sample rate.

3. The number of pericoronitis patients under the twenty-six years (young age) group is more than in the older age groups.

4. There is a significant increase in the concentration of (IL-8 and TGF-B1) in the saliva of patients with pericoronitis in comparison to the control group and also there was a positive correlation between those two markers.

6.2 Recommendation

It is recommended to:

1. Study the virulence factors of bacteria associated with pericoronitis infection such as bacterial biofilm.

2. Study other microbes causing pericoronitis infection such as viruses e.g. (Epstein bar, herpes virus) and fungi e.g.(candida).

3. study the relationship between the type of oral bacteria and genetic polymorphism of IL-8 and TGF-B1.

4. The same study with assessment of immunological markers and bacteriological findings after taking medicine or surgical treatment.

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