University of Babylon
College of Medicine

Bacteriological Study on *Streptococcus mutans* Associated with Dental Caries and Periodontal Diseases in Hilla City

A Thesis

Submitted to the Council of the College of Medicine - University of Babylon In Partial Fulfillment of the Requirements For the Degree of Master of Science In Medical Microbiology

By

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دراسة بكتريولوجية عن المكورات المسبحية الطافرة المتعلقة بتسوس الأسنان وأمراض اللثة في مدينة الحلة

رسالة

مقدمة الى مجلس كلية الطب/جامعة بابل

كجزء من متطلبات نيل درجة الماجستير

في علم الأحياء المجهرية الطبية

من قبل

فاطمة مالك عبود الربيعى

بكالوريوس طب وجراحة الفم والأسنان(2003)

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الخلاصة

تَضمّنتْ هذه الدراسةِ 90 عينةِ جَمعتْ مِن ْالمرضى الذين يعانون من تسوس الأسنانِ وامراض اللثة حيث أَخذتْ 50 مسحة مِنْ تسوس الأسنانِ و40 مسحةِ مِنْ حالات امراض اللثة لمرضى ادخلو إلى العيادات الاستشارية في كلية طبِ الاسنان في جامعةِ بابل والمركزالتخصصي لطب الاسنان في مدينةِ الحلة. هؤلاء المرضى كَانوا من كلا ألجنسين،و كانت أعمارهم تَتراوح بين 15-70 سَنَة.

أشارتْ النّتائِجُ الى أنّ، أكثر فئة ِعُمرية كانت معرضة لتسوس الأسنانِ هي (15-25) سنةً في حين الفئات العمرية ْ بعمر أكثر من 50سَنَةكانت اكثرتعرضا لامراض اللثة.

اوضحت النتائج بأنّ داء السكّري والتدخين لهما إرتبطا بتطورامراضِ الثة اكثر مِنْ ارتباطهما بإصابات تسوس الأسنانِ.

بينت النتائج ان من بين تلك التسعين عينة حوالي 86 من العينات كانت ايجابية لنمو البكتريا مقابل 4 نتائج سلبية الزرعَ. وقد تم عزل وتشخيصَ 24 عزلةْ ُ من بكتريا *S t.mutans* باستخدام الفحوصات الزرعية والبايوكيمياوية في حين لم يتم عزل هذة البكتريا من الاشخاص غير المصابين .

درست بعض عوامل الضراوة لهذة البكتريا وقد وجد ان جميع العزلات كانت منتجة للهيمولايسين نوع ألفا، بينما كان (35.7 %) منها قادرا على انتاج إنزيمات البروتيز الخارجية َ.

تم دراسة تأثير بَعْض المضادات الحيوية على عزلات *St. mutans* ، وقد اظهرت النّتائِجَ بأنّ اعلى نسبةَ مقاومة كانت لمضاد erythromycin (71.2%) وtetracycline وdoxycyclin كَانا المقاومةَ لكلاهما (69. 3 %). بينما كانت العزلاتَ مقاومة بدرجةِ أقلِ لكل من cefotaxime (38 %) وإلى ampicillin (25%) وamoxicillin (21 %) بينما كانتُ لَها نسبةُ مقاومةِ منخفضةِ جداً إلى amoxiclave (9.5 %)و ampiclox (% 14) و ciprofloxacin(17.3 %).

تم اختبار قدرة هذه البكتيريا لإنْتاج انزيم dextranase وقد وُجِدَ ان (33.3 %) من هذة البكتريا لَها القدرةُعلى إنْتاج انزيم dextranase الخارجية . كما تم اختبار تأثيرَ إضافةً بَعْض المضادات الحيوية على انتاج dextranase،وقد كَشفتْ النّتائِجَ بأنّ اعلى تثبيط لانتاج هذا الانزيم كَان مِن قِبل tetracycline(75 %) وتأثير التثبيط الأوطِأكَان مِن قِبل amoxiclave و ampicillin بنسبةِ تثبيط (38.5 %) لكلاهما والىcefotaximeبنسبة (25%).

و أخيراً كان معدل تركيزالكلوبينات المناعية(sIg A) من لعاب المرضى المصابين بتسوس الأسنانِ هو(13.8 mg/ مليلتر) بينما كان معدل التركيزَ لهذة الكلوبلينات في لعاب الا شخاص غير المصابين (8.9 mg / مليلتر). أي ان معدل تركيز هذة الكلوبينات في لعاب المرضى المصابين بتسوس الاسنان هو اعلى من معدل تركيزة في لعاب الاشخاص غيرالمصابين بتسوس الاسنان.

بسم الله الرحمن الرحيم

((قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا

إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ))

صدق الله العلي العظيم

سورة البقرة

الآية (32)

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List of Abbreviations

Symbol	Description
DW	Distilled water
DM	Diabetes mellitus
SIg A	Secretory immunoglobulin type-A
GTFs	Glycosyltransferase
PEG	Poly ethylene glycol
GbpB	Glucan binding protein B
PBPs	Penicillin binding proteins

Abstract

This study included (90) samples are collected from patients with dental diseases (50) swabs taken from dental caries and 40 swabs from periodontal cases ,who have admitted to collage of Dentistry in Babylon university and special center of Dentistry in Hilla city. These patients were of both sexes, their ages ranged from 15-70 years. Ten swabs taken from healthy persons as control.

The results indicated that, the most susceptible age group were(15-25) years old for dental caries and more than 50 years old were susceptible to periodontal disease .

The results revealed that diabetes mellitus and smoking were associated with development of periodontal disease more than their association with dental caries.

The results of bacterial culture were positive in 86 patients. versus 4 patient revealed negative bacterial cultures . It was found that 24 isolates(23.5%) were identified as *St. mutans* , whereas no isolates of *St. mutans* were found in the healthy control.

The virulence factors of *St. mutans* isolated in this study were studied and it was seen that all isolates were alpha-hemolysin

producers ,whereas (35.7%) of *St. mutans* produced extracellular protease.

The effect of some antibiotics on *St. mutans* was also tested, and the results showed that all isolates showed high resistance rate to Erythromycin (71.2%) and (69.3%) of isolates were resistant for both of Tetracycline and Doxycyclin. Some isolates showed lesser degree resistance to Cefotaxime (38%) , Ampicillin (25%) and amoxicillin(21%) , While these isolates were highly susceptible to Amoxiclav (90.5 %) , Ampiclox (86%) and Ciprofloxacin (82.7 %).

The ability of this bacterium to produce dextranase was also tested and it was found that (33.3 %) of *St. mutans* isolates have the ability to produce extracellular dextranase enzyme.

The effect of additions of some antibiotics on dextranase-producing culture was also tested and the result revealed the highest inhibition effect were by Tetracycline (75%) and the lowest inhibition effect was by Amoxiclave and Ampicillin with inhibition rate (38.5%) for both of them and Cefotaxime (25%).

Finally the concentrations of salivary sIg A were investigated . It was seen that the mean concentration of sIg A in patients with dental caries infection (13.8 μg /ml) was higher than those of healthy control (8.9 μg /ml) .

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I would like to express my thanks to **"Allah"** the Most Gracious and Most Merciful, and to his prophet "**Mohammad**".

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I extend my thanks to collage of Dentistry of Babylon university.

Fatima

Appendix (1)

Morphological and Biochemical Features for Identification of *St. mutans*

Test	Result
Growth on blood agar aerobically and an aerobically	Positive
Catalase	Negative
Oxidase	Negative
Heamolysis	α –heamolysis
Gram stain	+ ve
Shape of cells	Cocci ,chain
Esculin hydrolysis	Positive
Motility	Negative
Mannitol fermentation	Positive
Glucose =	Positive
Sucrose =	Positive
Raffinose =	Positive
Vancomycin sensitivity	Sensitive
Growth in 6.5 %Nacl broth	No growth

Appendix (2)

Morphological and Biochemical Features for Identification of Bacterial Isolates from Dental Infection.

Test	Gram stain	Catalase	oxidase	hemolysis	coagulase	Motility	Growth on mannitol
Nisseria spp.	G-ve dipplo cocci	-	+	Gamma	/	_	/
S. aureus	G+ ve cocci(cluster)	+	-	Beta	+	_	Golden
S. epidermidis	G+ ve cocci(cluster)	+	_	Gamma	_	_	White
Lactobacilli	G+ve Rod (bacilli)	_	/	/	/	_	/
Actinomyces	G+ve branching (filamentous)	/	/	/	/	_	/
St.salivarius	G+ve cocci (strep.)(aerobe)	_	_	Gamma	/	_	/
*St.pyogen*es	G+ve cocci (strep.) (aerobe	_	_	Beta	/	_	/
Peptostrep.	G+ve cocci (strep.) (anaerobe).	_	_	_	/	_	/
Veillonella spp.	*G-ve anaerobe cocci*	/	/	_	/	–	/

Appendix (3)

Concentrations of Immunoglobulin (sIg A) (µg/ml) of patients and Its Optical Density

The final concentration	Optical density
11.55 µg / ml	0.127nm
18.46 µg / ml	0.190nm
17.43 µg/ml	0.141nm
16.97 µg/ml	0.185nm
17.62 µg/ml	0.192nm
17.25 µg/ml	0.188nm
12.11 µg/ml	0.133nm
11.17 µg/ml	0.123nm
10.24 µg/ml	0.113nm
18.46 µg/ml	0.201nm
13.77 µg/ml	0.151nm
10.80 µg/ml	0.119nm
11.55 µg/ml	0.127nm
14.63 µg/ml	0.162nm
10.52 µg/ml	0.116nm
10.99 µg/ml	0.121nm
12.33 µg/ml	0.132nm

Certification

We certify that this thesis was prepared under our supervision at the Department of Microbiology, College of Medicine, University of Babylon as partial fulfillment of the requirements for the Degree of Master of Science in Medical Microbiology.

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In view of the available recommendation, I present this thesis for evaluation by the Examining Committee.

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Conclusions and Recommendations

Conclusions

1. Oral cavity is colonized by large number of microorganisums that contribute in dental diseases. *St. mutans* considers principle etiological agent of dental diseases that appear in (23.5%) of total isolates

2. All *St. mutans* isolates show very low level of resistance to amoxiclave (9.5 %) and ampiclox (14 %) and high level of resistance to erythromycin (71.2 %),followed by tetracycline and doxycyclin (69.3 %) .

3. It is observed that (35.7 %) of *St. mutans* isolates produced extracellular protease .

4. It is found that (33.3 %) of *St. mutans* produced dextranase enzyme extracellularly. And the highest inhibition effect on dextranase production by antibiotics was recorded by Tetracycline (75%) , while the lowest inhibition effect was recorded by cefotaxime (25 %).

5- The mean concentration of sIg A in patients with dental caries infection (13.8 µg /ml) were higher than those of healthy control (8.98 µg /ml) .

Recommendations

According to the results obtained in the present study , we can recommend the following :

1. Performance of epidemiological survey for *St. mutans* infections.

2. Prevention of long term use of antibiotics which effect on normal flora and then facilitate the colonization of other pathogenic bacteria.

3. Further studies are required for *St. mutans* at the molecular level.

4. Study the possible use of dextranase as preventive measures (antiplague agent) in dental practice.

5. Investigation the role of immune system against dental infection.

Dedication

To the Daughter of the Holy Prophet and the Wife of

Imam Ali

Fatima Al-Zahra

Leader of the women of paradise

And To my mother, my father .....

To my husband Dr.Haider & my daughter
Zainab my son Hussain
To my brothers ,my sisters with my endless love

Examination committee

We, the examiner committee, certify that we have read the thesis entitled **(Bacteriological Study on *Streptococcus mutans* Associated with Dental Caries and Periodontal Diseases in Hilla City**) and have examined the student **(Fatima Malik Aboud Al- Rubiae)** in its contents, and that in our opinion it is accepted as a thesis for degree in Master of Science in **Medical Microbiology** with excellent estimation.

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Introduction & Litrature Review

1.1 INTRODUCTION

Dental diseases are one of most common human infections ,and considerd as a major public health problem .They occur in number of ways ; via the introduction of pathogens of extra-oral origin ; through change in the balance of the indigenous flora and by entry of bacteria in to the sterile vital pulp of the tooth(Loesche,2000) . They include two major diseases which are dental caries and periodontal disease .(Anderson,2004) .

Dental caries is a major infections disease that damage the hard tooth structures ,it causes localized destruction of the enamel , dentin and / or cementum of the tooth by acid produced from the bacterial degradation of fermentable sugars (Todar , 2008) .The resulting acidic levels in the mouth will affect tooth because tooth is special mineral content makes it be sensitive to low pH (Anderson,2004 ; Ash, and Nelson, 2003)

Clinically , it can be classified into pit and fissure , smooth surface and cemental caries ,it effect the crown and root of tooth . Root caries is commonly seen in elderly people .(Rosentiel, and Stephen, 2000).

Other dental diseases are periodontal diseases which refer to group of problems that arise in sulcus (crevice between gum and teeth) .It is the bacterial infection that affect the supporting structures of teeth

(gingiva ,cementum , periodontal membrane and alveolar bone). It is associated with accumulation of bacterial plaque (Loesche ,2000) .

Two events occur in the oral cavity lead to development of periodontal diseases : increase in the quantity of anaerobic G-ve bacteria and shift in the over all balance of bacteria from harmless to disease causing one (Darling, 2007).

The most important causative agent for dental caries is S. mutans bacteria .Because its activity which lead to colonization of the tooth surfaces , plaque formation and localized demineralization of tooth enamel , (Todar ,2008) .However it is not only cause of dental decay , but ,various oral bacteria gain access to interior region of the tooth such as , Lactobacilli and *Actinomyces* which considered as secondary invader that contribute to progression of lesions .(Ash and Nelson,2006) .

Cariogenic *St, mutans* adhere to tooth surface by synthesis of extra cellular polysaccharide (Dextran) from sucrose ,but when the sucrose not available in the mouth ,it will produce the dextranase enzyme that decomposed the dextran matrix of dental plaque as carbon source .So it still produce lactic acid that dissolve tooth structures.(Igarashi ,*et al.*, 2004) .

Among the bacteria most commonly implicated in periodontal diseases are *Actinobacillus. ,P. gingivalis* , *Peptostreptococcus.* , f*usibacterium* and *veillonella* Spp. The mechanism of tissue

destruction in periodontal diseases are due to hydrolytic enzymes ,endotoxins and other toxic metobolites (Todar , 2008).

The treatment of dental diseases consist of identifying the cause , extent of infection and eradicating the infection and its cause. (Westerman ,*et al.*,2006).

Aims of the study

1- Description of the oral microbiota associated with dental diseases to identify the specific etiological agents.

2- Isolation and identification some of *St. mutans* isolates which are associated with dental caries and periodontal disease in patients aged between (15-70) year old from both sexes.

3- studying some virulence factors of *St. mutans* such as hemolysin production , extracellular proteases and antibiotics resistance.

4- Detection of dextranase producing *St. mutans* and studying the effect of some antibiotics on dextranase production.

1.2 Literature Review

Oral cavity is open growth system , in which the bacteria must be adhere to surface in order to produce colonization which is necessary prior to any odontopathic or periodotopathic process .(Liligemark and Bloomquist,2002). The oral cavity is colonized by more than 300 - 400 species of aerobic and anaerobic bacteria but only limited number of these species participate in development of dental infection.(Loesche,2000).

The presence of nutrient ,epithelial debris ,saliva secretion make the mouth a favorable habitat for great variety of bacteria . Odontogenic infection are caused by shift in the behavior and composition of plaque bacterial communities .(Peterson,*et al.,* 2002) .

Dental caries is the bacterial disease of the dental hard tissue . It characterized by the irreversible solubilization of tooth mineral by acid produced by bacterial communities known as dental plaque.(Bradshaw and marsh,1998) .

Other dental disease is the periodontal disease ,which is inflammatory response to dental plaque bacteria ,occur in periodontium of tooth . In general it divided into two groups which are gingivitis (infection of gum) and periodontitis is the infection that damage the alveolar bone and supporting structures of tooth .(Moore,1994).

1.2.1 Tooth Anatomy:-

The tooth is the hard structure that composed from three layer : inner most layer is the pulp which contain the nerve and blood vessels,is responsible for tooth sensation, middle layer is dentin region that enclosed the pulp region and third layer is the enamel of tooth that cover the dentin in crown part of tooth above the gum line and the cementium that cover the dentin in root region.The visible part of tooth is called the crown and the embedded part is called root.(Berkowiz , *et al* .,1992) .(Figure 1-1).

1.2.2 Periodontium Anatomy:-

The periodontium is the supporting structure of the tooth that is composed of gingiva , gingival crevice , periodontal ligament , root surface and alveolar bone (Berkowiz ,*et al*.,1992 ; Loesche,2000). Figure (1-1).

1.2.3 Predisposing Factors (Risk factors):-

1.2.3.1 Age:-

The onset of caries in susceptible people is early ,it usually occurs after the eruption first deciduous or permanent teeth ,within short period of time , Russel ,(1989). The incidence of new lesion remains high during period of puberty and adolescence(Greene ,*et al.*,1987) and for that reason dental caries has been described as disease of

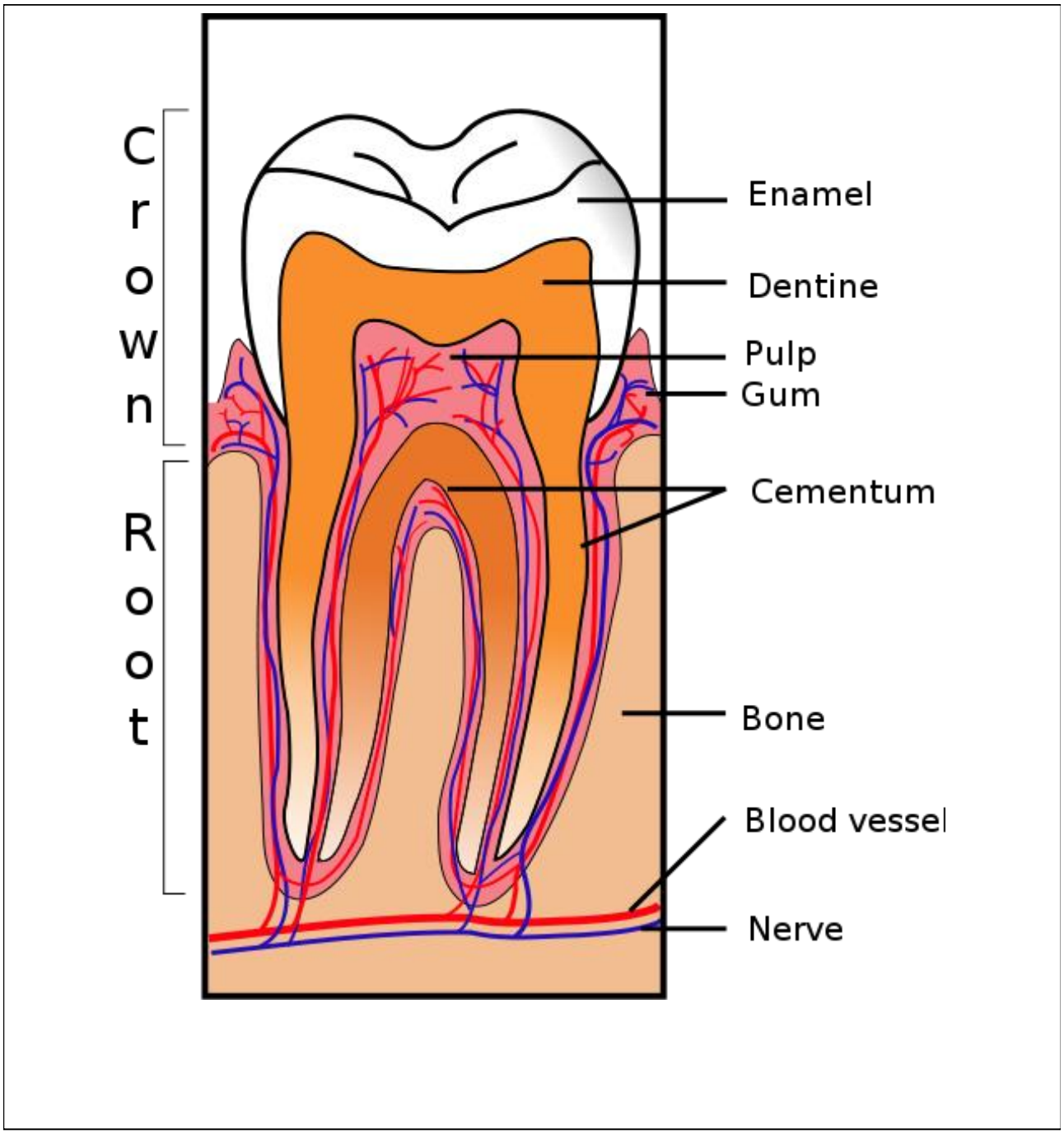

Figure (1-1) anatomy of the tooth (from Loesch,2000)

children. Berman and Slack , (1989), have demonstrated that the caries activity among children aged 11-15 years old increased sharply.Another study show decline in caries activity following adulthood (Burt,*et al.*,1981). Factors such as change in dietary habits and buildup of fluoride in outer layer of enamel make the tooth resist for dissolving by acid. About the age and periodontal disease many studies reveal that the prevalence and severity of periodontal disease will progress steadily with age (Hugoson ,1980 and Russel ,*et al.*,2000). One survey reported that 14 % of 34 year old have sign of gingivitis ,the same study 86 % of more than 50 year old had periodontitis .(Loesche ,2000). However ,tooth loss due to periodontal disease before the age of 20 year is rare (Soory,2002). The possible reason for that dental disease worsens with age is due to the fact that the host defense will decrease with age (lo¨e,*et al.*,1988) .

1.2.3.2 Sex:-

Numerous studies had correlate dental disease with sex. Many epidemiologic studies were conducted to assess prevalence and severity of dental disease had shown that it is more prevalent and sever among females than males (Yanover and Etlen, 1980 and Anderson ,*et al.*,2006). However, Beiklere ,*et al*., (2002) had reported no significant differences in dental disease between males and females.

The susceptibility of females to dental disease is due to change in hormone level during period of pregnancy ,menstrual cycle and menopause will predispose to dental infection .(Robison ,*et al.*, 2000).

1.2.3.3 Diabetes Mellitus:-

Diabetes mellitus (D.M) is complex metabolic disease that predispose for many dental infection such as dental caries and periodontal disease(Ueta,*et al.*,1993). There is good relation between dental infection and poorly control D.M especially in the patient with of periodontal disease. Diabetic children have more sever gingivitis than healthy one .(Oliver and Tervonen ,1994) .

The possible reasons for the increased susceptibility of D.M patient to periodontal disease could be due to changes in vascular and polymorph nuclear leucocytes (PMN) function, so it effects on host response to dental infections. The changes in capillary basement membrane could have an inhibitory effect on the transport of oxygen, W.B.C and immune factor, all of which could effect on the defense mechanism and tissue repair.(Salvi, *et al.*,2000). While impairment of PMN in D.M patient include reduce phygocytosis and chemotactic responses, so this lead to development of periodontal infections .(Piwowar,*et al*.,2000) .

1.2.3.4 Smoking:-

Smoking is most significant risk factor for dental disease because it lead to damage the immune system , creation favorable environment for harmful bacteria and impairment the healing process ,(Ah ,*et al.*,1994). A Clear association between smoking and prevalence of dental disease were reported by Kowalik and Nisbet,(1983) . A study by (Macgregor,*et al.*,1999) have also indicated that smoking is important predisposing factor in development of dental disease especially periodontal disease.

Similarly , Falklers ,(1998) ,in a study of 135 periodontitis patients in urban dental school ,had found that 83 % of them were smokers. Smoker tend to have poorer levels of oral hygiene and greater deposits of calculus than non smokers .Its effect on local and systemic host response due to the smoking may causes vasoconstriction of periodontal blood vessels and may in this way favor the colonization of anaerobic bacterial flora (Totti ,*et al*.,1994 and Ryder ,1996).Also it causes decrease in serum Ig G , and T lymphocyte against subgingival flora, and it cause neutrophil function impairment. (Costable ,1994).

1.2.3.5 Use Of Drugs:-

The use of drugs such as anticonvulsant ,antidepressant and antihistamine will decrease production of saliva that act as defense against harmful bacteria,(Sooriy *et al*.,1990). Other drug which

causes suppression of immune system, also predispose to dental infection(Bots,*et al.*,2004).

1.2.4 Dental Caries:-

Tooth decay is the infectious diseases which damage the structure of teeth. The sequences of caries are the cavities, if left untreated it can lead to pain, tooth loss, infections of underlying structure and in sever case causes endocarditis. (Nelson,*et al.*, 2000).

In 1890,W.D.Miller conducted a series of studies that led him to propose an explanation for dental caries because he found that *St. mutans* produced acids which dissolved tooth structure in presence of fermentable sugar,(Kleinberg,2002). This theory known as chemo-parasitic theory ,it consist of two stages first one is decalcification of enamel and dentin followed by dissolution of the softened residues ,(Kozai, *et al.*,1999).

Two other theories explaining the caries process are the proteolytic theory and the proteolysis –chelation theory .(Ko¨hler,*et al.*,1995).

Anderson,(2004) had indicated when the carious lesion pass through enamel, dentinal tubules and then pass to the pulp the tooth became exposed and hurt, the pain can be worsened by heat and cold. When the infection can spread from tooth to bone and surrounding soft tissue it will causes osteomylitis and loud wig angina respectively .(Kleinberg,2002)

1.2.5 Periodontal Disease:-

Periodontal disease are bacterial infections that affect the supporting structures of the teeth (gingiva, cementum , periodontal membrane, and alveolar bone.(Loesche, 2000). It consist of gingivitis, periodontitis, acute necrotizing ulcerative gingivitis (AUNG).(Klein,*et al.*,2000). Diseases that are confined to the gum don't lead to loss of teeth, but there are other more serious forms of periodontal disease that affect the periodontal membrane and alveolar bone resulting in tooth loss.(Slote,1986).

The bacteria associated with these lesions are very complex populations consisting of *G* +ve organisms (including *Actinomyces spp*. and *Streptococci spp*.) and *G* –ve *organisms*(including *Spirochet spp., Veillonella spp.* and *bacteroids spp*.).(Todar, 2008).

The most common form is gingivitis , is inflammatory conditions of gums. It associated with increase population of *Actinomyces* that accumulate in plague around the tooth.(Winkehoff, *et al.*,2002).

The periodontal disease can also associated with a number of systemic diseases as predisposing factors, including type 1 diabetes, Down´s syndrome, AIDs and several blood disorder in WBC.(Loesche, 1986). Stress and smoking are also contribute in development of this disease.(Ryder,1996).

Among the bacteria most implicated in bone loss were *Actinomyces* spp. , *Actinobacilli* spp., *Porphyromonas gingivalis. P. gingivalis* doubles the risk for serious periodontal disease ,because it may release specific proteinase that destructive and disrupt the immune the immune system and lead to subsequent periodontal tissue destruction.(Ah, *et al.,* 1994). Other bacteria associated with this disease is *Treponema denticola* (Listgarten, *et al.*,1991).

Zambon ,(1996) ,had indicated that abnormal oral environment include lack of oral hygiene and frequent sugars intake. The bacteria that causes periodontal disease thrive in acidic environment because increases the acidity in the mouth will increase the bacterial counts.

1.2.6 Microbial Etiology of Dental Infections:-

Microorganisms colonize in dense mass forming dental plaque on teeth. Dental plaque consist of microbial communities organized in a complex matrix composed of microbial extrcellular polysaccharide (dextran) and salivary compounds. The microbial composition of dental plaque varies according to the site and sampling time.(Shafer, *et al* .,1993). Dental plaque develops preferentially on teeth surfaces protected from mechanical friction, such as the area between two teeth (a proximal surface , sub gingival areas (gingival crevice) and the pit and fissure of the biting surfaces Lindquist and Emilson, (1990). Dental plaque is acomplex microbial biofilm, it is considered as the primary etiologic factor in development of dental caries and

periodontal disease. The bacteria form (60%- 70%) of its volume , may reach to 300 -500cell. These accumulation subjected teeth and the periodontium to high concentration of bacterial metabolites ,which result in dental infection. Plaque bacteria produce two form of toxin which work as extracellular enzymes and endotoxin (Shimotoyodome, *et al.,* 2007).

St. mutans are primary colonizer of oral hard and soft tissue. It is highly cariogenic because it ferment dietary sugar (sucrose) to lactic acid , which is dissolving tooth structures and lead to cavity (Anderson, *et al.,* 2006).

Cross section and longitudinal studies have shown strong relationship between the presence of *St. mutans* and intiation of lesions. This association is the strongest in fissure caries ,while in advanced lesions there are high proportion of Lactobacilli.(Todar,2008). Root surface caries are caused by *Actinomyces spp*. ,in addition to *St. mutans* and Lactobacilli bacteria .

The most common causative agent bacteriologically is *St. mutans* ,which is G +ve bacteria, spherical that form pairs or chains during the growth , some are member of normal flora of the mouth.(Brooks, *et al*., 2007). It is belonged to viridians Streptococcus *(St. mutans, St. sangius, St. salivaius,* and *St. mitis).(*Loeshe,1986).

Mutans Streptococci *(St. mutans , St. sobrinus, St. cricetus and* St. *rattus)* and *St. sangius* are found in large number on teeth ,while *St.*

salivarius is isolated mainly from the tongue. (Whiley and Beighton,1998). *St. mutans* and *St. sangius* appear in the oral cavity only after eruption of the teeth .(Loeshe,1986 and Kozai, *et al.*, 1999).

The major route for early acquisition of *Mutans streptococci* is vertical transmition from mother to child.(Kozai , *et al.*, 1999).

St. mutans are predominant spp. isolated from human saliva and play essential role in the development and progression of dental caries (Saini,*et al.*,2003).

1.2.6.1 *St. mutans* and Dental Infections :-

St. mutans is first described by J .Killin in 1924, in deep dentin carious lesion and dental plaque samples. He found a small chained coccobacillus which is more oval than spherical in shape. He suggest that these microorganism are Mutans Streptococci and called them *St. mutans.*

St. mutans occur in pairs or in short or medium length chains ,with out capsule .Under acid conditions in broth and on some solid media, these cocci may form short rods 1.5-3.0 μm in length . Rod shape morphology may be evident on primary isolation from oral specimens (Marsh and Martin, 1999).

St. mutans require nutritionally rich media for growth .The metabolism is fermentable producing mainly lactate but no gas. They

are catalase negative and they commonly attack red blood cell (Nolte, 1982).

Many studies have shown that *St. mutans* is regularly isolated from incipient or well developed carious lesions , but less commonly from dental plaque around tooth surface.(Saini,*et al.*,2003) .

In alongitudinal study, it is found that *St. mutans* level in plaque increases 6 -24 months before the clinical appearance of dental caries.(Whiley and Beighton, 1998).

Increases in the proportion of *St. mutans* are observed to occur before the lesion became active ,(Marsh and Martin , 1999). However it is noted that sound enamel may some times be colonized with a relatively high number of *St. mutans* ,(Hardie, 1986). and that some caries free populations have high plaque count of *St. mutans* in these situations ,caries may not have developed either because the teeth are relatively resistant to acid attack or because the individuals do not have a particularly cariogenic diet (Sissons, 1997).

Studies using broadly active plaque control agent such as chlorhexidine provide further evidence of the association of *St. mutans* and dental caries in that regular application can lead to significant reduction in the levels of both *St. mutans* and dental disease incidence (Binney *et al.,(*1996).

Further evidence for *St. mutans* involvement in the etiology of dental disease especially caries had came from immunization studies.

In a study the oral administration of *St. mutans* cell to gnotobiotic rats induce the production of secretory IgA in the saliva and this correlate with the a reduction in disease incidence in these animals.(Kozai, *et al.,* 1999). In another study , intravenous administration of *St. mutans* cells to these rats led to serum antibody response and an associated decrease in dental plaque incidence.(Mattos ,*et al* .,2001).

1.2.6.2 Virulence Factors of *St. mutans*:-

Virulence factors of *St. mutans* help to protect the bacterium against possible defenses and maintain its ecological niche in the oral cavity. Probable virulence factors include : adhesions , acid production ,acid tolerance, protease production and production of glucosyltransferases and intracellular polysaccharides .(Kuramitsu,1993) .

Product of other gene that may also contribute to the virulence of *St. mutans* such is hemlysins.(Ajdic,*et al.*,2002).,

The physiological and biochemical properties which implicate in virulence of *St. mutans*: it ready colonizes tooth surface by binding to enamel pellicle ,(Todar ,2008) ,it contain glycosyltransferase that act as bacterial ligand for attachment and produce lactic acid from the utilization of dietary sugars.(Park, 2000).

1.2.6.2.1 Colonization of Tooth :-

St. mutans is aregular component of oral normal flora ,which ready colonize tooth surface. The adhesion of S. mutans is virulence factor with function critical to viability ,(Nakai,*et al*,1993).

Adhesions are found as cell wall component or associated with cell structures such as fimbriae . The receptors may be salivary components (mucins ,amylase , Ig A, Ig G and prolin –rich protein) or bacterial components (glycosyltransferase and dextran) .(Moisset, *et al.,* 1994).

Acidogenic Streptococcus requier the hard tooth surface for sustained colonization accumulation. Initial attachment to the tooth is achieved via the interaction of bacterial proteins with lectins in dental pellicle covering the tooth surface.(Bradsh and Marsh, 1998).

1.2.6.2.2 Acidogenicity and Acid Tolerance:-

St. mutans ferment many different sugars and they metabolize sucrose to lactic acid more rapidly than other oral bacteria (Nishi, *et al.,* 2002). This metabolic reaction renders the dental plaque acidic in presence of fermentable carbon source. Acid tolerance of *St. mutans* enable them to continue metabolism even at low pH.(Mattos,2001). It had been demonstrated that strains of *St. mutans* are more acid tolerant than another bacteria examined with exception of Lactobacilli.*(*Loesche ,2000).

The property of acid tolerance (or acidurance) appear to be connected with the membrane associated proton –translocating ATPase of these organisum.(Bradshaw and marsh , 1998).

1.2.6.2 .3 Intracellular Polysaccharides:-

Most strains of *St. mutans* produce intracellular iodine-staining polysaccharide (IPS).(Makinen,1990).

According to results of experimental studies with rats that some strains of *St. mutans* that are highly virulent in rats are capable of synthesis dextran polysaccharide ,whereas other that are not synthesis (IPS) were avirulent , suggested another potential virulence property of these organisms.(Kuramitsu, 1993).

Because of (IPS) storage ,these cariogenic bacteria have ability to continue fermentation in absence of exogenous sugars supplies. (Todar, 2008).

1.2.6.2.4 Bacterial Hemolysin:-

Hemolysin is hemolytic protein produced by some bacteria. The molecular structure of Hemolysin is different from one bacteria to another. Its production is mostly associated with pathogenic bacteria .(Whiley and Beighton,.(1998). It plays a role in blood invasion and in supplying the bacteria with their requirement of iron.(Hardie,1986).

Two types of hemolysin in *St. mutans* strains have been described. The first is an oxygen labile hemolysin(Streptolysin O). The second type is an oxygen stable hemolysin (Streptolysin H).(Marsh and Martin, 1999). It is found that genes coded for hemolysin may be present on conjugant plasmids or on non conjugant plasmid.(Shafer ,*et al.*,1993).

1.2.6.2.5 Extracellular proteases:-

One of suggested virulence factors is the protolytic activity of mutans streptococci.(Gazie, *et al.*,1997).

St. mutans has been shown to produce two extracellular proteases, capable of degrading both gelatin and collagen-like substrates.(Curtis, *et al.*,1999).

St. mutans proteases contribute to virulence and are involved in the breakdown of host proteins for bacterial nutrition and the direct degradation of host structural proteins. These streptococcal proteases may also be involved in the destruction of some components of the host immune system.(Colby and Russell, 1997). Many oral streptococci produce sIgA protease, which impairs the host defense by cleaving the sIgA.(Hamada, *et al.*,1989).

1.2.6.2.5 Glycosyltransferase Enzymes:-

St. mutans synthesize glycosyltransferase(GTFs), which serve as bacterial ligand for attachment because it catalyzes polymerization the glucose which derived from dietary sucrose to dextran, later directly lead to the formation of dental plaque.(Kopec, *et al.*,1997).

St. mutans GTFs are consist of 1500 amino acid long and have two functional domains, the amino terminal portion is catalyze cleavage of sucrose and carboxyl terminal portion which catalyze dextran binding.(Ooshima, *et al.*, 2001).

The glucose polymers provide scaffolding for the aggregation of mutans and other oral streptoccoci through interaction with bacterial cell associated glucan binding proteins.(Mattos, *et al.*, 2001).

1.2.6.3 Dextran-Hydrolysing Enzymes:-

St. mutans, which causes dental caries adhere to tooth surface through the synthesis extracellular polysaccharide(dextran) from dietary sucrose(Shimotoyodome,*et al*.,2007). Dextran is homoglycan of α-D glucose molecule bonded by α-1,6 and α-1,3 glycosidic bond . (Khalikova,*et al.*,2005).

Dextranases are dextran-degrading enzymes form diverse group of different carbohydrases. These enzymes have often been classified as endo and exo dextranases based on the mode of action and commonly called dextranases.(Garcio and Rodriguez,2000). It is also

classified according to type of reaction in to dextran α -1,6 glycosidases , α-1,3 glycosidaes and dextran α- 1,6 and 1,3 isomaltotriosidaes .(Garcio and Rodriguez,2000).

Oral streptococci are predominant producers of dextranase. Strain of *St. mutans* costitutively and induciblely produce both endo and exodextranases.(Khalikova, *et al.,* 2005). Endodextranas is mostly cell associated and its activity provide primer or branch points for glycosyltransferases and thus contribute to the complexity of dextran structure.(Colby and Russell,(1997) and Abdel – Naby ,*et al.,* 1999).

Wider range of oral species associated with dental plaque produce inducible dextranase extracellularly like *Bacteriod* spp., *Bifidobacterium* spp., and *Fusobacterium* spp.(Ajdic, *et al*., 2002). *St. sobrinus* and *St. salivarius* don't have such mechanism and are unable to utilize the dextran as sole carbon source . while, *St. mutans* break down the dextran polymer in dental plaque.(Simonson, *et al*., 1994).

The current knowledge of sugar metabolism of *St. mutans* strain, suggest that this organism is capable of metabolizing a wider variety of carbohydrates than any other *G* +ve bacteria and thus, carbohydrate metabolism is the key survival strategy for *St. mutans*.(Colby, *et al*., 1995).

These enzymes can depolymerize various microbial dextran deposite, the fact that dextran is acomponent of dental plaque which

contribute to development of dental infection.(Igarashi, *et al.*, 2002). So recent application of this enzyme in treatment of dental plaque through degrade and remove the dextran which form 20 % of the dry weight of dental plaque.(Khalikova, *et al*.,2005). Using of dextranase have been suggested to prevent oral diseases such as dental caries and periodontal diseases. These *in vitro* properties of dextranase are considered this enzyme important anti plaque agents.(Simonson, *et al*., 1994).

Another approach to the control of dental plaque would be genetic engineering oral commensal organism to antagonize the cariogenicity of *St. mutans* strains. The genes encoding dextranase have been cloned and expressed in oral streptococcus , so the transformant *St. gordonii* have been repress firm adherence of dextran polymer form by *St. mutans* in dental plaque.(Colby, *et al.*, 1995).

1.2.7. Immunology:-

The oral cavity is the site of secretory immune system similar to those of other areas of respiratory tract. Local and systemic immune responses occur in patient with acute or chronic dental disease (dental caries and periodontal diseases) .Immunolgically active antigen interact with cell in lamina properia to produce local immune response like sIgA ,in other ward the saliva of patient with dental diseases contain all class of Immunoglobulins (Klein,*et al*.,2000).,mucos layer of oral cavity contain several substance having

antibacterial effect like lactoferin; lysozyme ; fibronectin ;lactoperoxidase (Stenfors,1999). Other mechanism of defense in mouth is presence of saliva and its mechanical washing of infection .(Jean-sanchia,*et al.*,2001)

1.2.7.1 Humeral immunity in oral cavity:-

In oral cavity the mucosal immune system is composed of the lymphoid tissues that are associated with mucosa of mouth. It has evolved during an antigenic exposure early after birth . It has anumber of features that differentiate it the systemic lymphoid tissues, these include the production of secretory (sIgA) and population of T cells. Mucosa specific regulatory properties, thus qualifies mucosal immune system, as some was separate immunologically from systemic immunity.(Strober and Fuss, 2001) .

The humeral mucosal immune response is the first line of defence against pathogens encountered after ingestion of this pathogens, exerted through mechanical washing and by specific immunity .Antibody in saliva do not act as bactericidal ,but rather by binding to Ag, neutralizing , agglutination and immobilization of pathogen.

The principle Ab involved in the mucosal immunity is secretory IgA (Underdown and Mestecky ,1994),which is predominant immunoglobulin produced by plasma cell in sub mucosal lymphoid tissues ,thus, although IgA account for only 10-15 of serum Ig, it is by far the most abundant Ab class found and other secretion such as tears

; milk ;urine ;respirarory and bronchial secretions.

On B cell IgA exists as monomer(M.wt=160000dalton) comprising only one four-chain unit in secretion. It multimerizes to form disulfide linked polymer of up to five such units that are associated with one molecule each of J chain and secretory component, the predominant secreted form of IgA are dimmers (Parslow *et al* .,2001) .

1.2.7.2 Secertory IgA Function:-

When the pathogen adapted to infect tooth surface and mucosa,they express virulence factor that allow them to adhere and colonize tooth surface and mucosa. Secretory IgA prevents absorption of these bacteria and toxin by blocking their adhesion.. So it leads to prevent the infection. sIgA flushed away in steam of secreted fluids and mucous washing over the epithelial membranes .(Mazanec, *et al*.,1993).

During dental diseases ,secretion of IgA increased to prevent the infection by bacteria responsible for dental diseases .(Dunn-walters *et al*.,2003). Increased the level of salivary sIgA indicated to increase dental infection especially gingival inflammation ,in other ward during dental diseases, secretion of sIgA increased to prevent infection by bacteria responsible for dental diseases also it secreted due to presence of normal flora in the mouth ,this natural (Ab sIgA) act by cross reactive against the actual pathogen such as *St. mutans*.

A report by Nolt,(1982),showed that 68% decrease in sIgA in saliva noted in subject with acute necrotizing ulcerative gingivitis (ANUG) as compared with normal person. So decrease in concentration of sIgA could possibly result in increase pathogenicity of microorganism due to increase in number and metabolic products of pathogens

Lehner and co-workers(1996) demonstrated that sera from subjects with DMF (decayed, missing, filled) score had significantly higher Ab titer for cariogenic streptococci than sera from subjects with low DMF index. SIgA can bind to organism and result in agglutination of it and increase its phagocytosis ,so removed by saliva ; new approaches to prevention dental diseases specially dental caries .Thus done by vaccination with one of cariogenic streptococcus Ags .(Smith, *et al* .,1998).

1.2.8 Treatment of Dental Diseases:-

The treatment of dental diseases may include medical and surgical intervention or both ,depending to type of lesion and its severity , on patient condition and type of etiological agents(Anderson, 2004). Medical treatment include using local and systemic antibiotics and use of these antibiotics as prophylaxis against local infections.(Marron, *et al.*, 2001).

Sanchis,*et al.*, (2004), estimated that 10 % of all antibiotics were related with dental infection. In the same study ,they had been shown

that most frequent antibiotic prescribed by dentists was amoxicillin-clavulante.

The indication of use of antimicrobial therapy in dental practice includes following conditions acute ulcerative gingivitis , periodontitis ,periodontal abscess and dentoalveolar infections.(Teng, *et al*.,1998).

Povida, *et al*.,(2007), showed that the antibiotics frequently used in dental practice are Ampicillin ,Amoxicillin Tetracycline ,Doxycyclin ,Erythromycin and Trimethoprime.

Management of dental infections by topical application of antibacterial agent as mouth washes are considered effective measure to control dental infections.(Teng, *et al*.,1998 and Leistevuo,*et al*.,2000*).*

DeAzavedo,*et al.,(*1999). showed that the plasmids, phages and transposones contribute to transferring the genes which are responsible for the antibiotics resistance among bacteria.

In a study investigated the susceptibility of 424 isolates of *St. mutans* taken from 116 children and student ,all bacteria were found to be susceptible to penicillin as well as to Amoxicillin, Trimethoprime , Tetracycline and Erythromycins.(Leistevuo,*et al*.,,2000).

1.2.9 Prevention of Dental Disease :-

1.2.9.1 Oral Hygiene

Oral hygiene programs were indicated to minimize any etiological agents of infection by removing and prevention the formation of dental plaque.(Bots,*et al.*,2004).Thus, personal hygiene care consists of proper brushing and daily flossing to decrease chance of infections .(Baehni and Guggenheim,1996).

1.2.9.2 Dietary Modification

For dental health, frequency of sugar intake is more dangerous than the amount of sugar consumed .(Summit ,*et al.*, 2001). In presence of fermentable sugar, *St. mutans* in the mouth will ferments these sugar and produce acid which may demineralize tooth structure. Therefore minimizing sugar in take is recommended to decrease the oral disease.(Alyka ,*et al*.,2006).

Using modified diet such as chewing containing xylitol prevents dental plaque formation ,because the oral bacteria unable to utilize it. Also the chewing gum lead to increase production and release of saliva which act as natural buffer against the lowering of pH in mouth.(Smith, *et al.*,1998).

1.2.9.3 Other Preventive Measure

Fluoride therapy is recommended to protect from dental diseases by binding to hydroxyaptite crystals in enamel and increase its mineralization. It supplemented as water fluoridation and topical use as tooth paste or mouth wash.(Ross, *et al.*,2003 and WHO,2003).

Dental sealant is agood means of prevention. It act as a coat covering the biting surface so it leads to prevent of accumulation of plague in pit and fissure. Thus prevent pit and fissure caries.(Cate, 1998). Other recent research was suggested the use of vaccine for prevention of dental caries .(Ross, *et al.*,2003).

2.1. Patients

A total of (90) swabs were taken from patients suffering from dental diseases (50) swabs were taken from (26) males and (24) females with dental caries cases and(40) swabs were taken from (16) males and (24) females with periodontal diseases cases , who admitted to consultant unit of the collage of dentistry in Babylon university and also to consultant special center of dentistry in Hilla city, during a period extending from November 2007 to April 2008 .All patients involved in this study were distributed according to their age and sex .Predisposing factors such as smoking and diabetic state were also studied.

2-2 *Materials:-*2.2.1 Laboratory Instruments

No.	instruments	Company
1	Sensitive Electronic Balance	A and D, Japan.
2	Autoclave	Stermite, Japan.
3	Incubator	Memmert, Germany.
4	Distillator	GFL- Germany.
5	Centrifuge	Hermle, Japan.
6	Oven	Memmert, Germany.
7	Refrigerator	Concord, Italy.
8	Milipore Filter	Satorius membrane W. Germany.
9	Light Microscope	Olympus, Japan.
10	Micropipette	Oxford, USA.
11	pH Meter	Hoeleze and Cheluis, KG, Germany.
12	Water Bath	Memmert, Germany

2.2.2 Chemical and Biological Material

Company	Name of material
A- Chemical Materials	
MerkDarmstadt .	K_2HPO_4 , NaCl, $MgSo_4$, HCL, $CaCl_2$, Na OH, $BaCl_2$
B.D.H	Formalaldehyde,Tetramethyl-p-paraphylene diamine dihydihydrochloride, trichloroacetic acid, ethyle alcohol, Phenol red , Chloroform, gelatin, peptone, ferric amonium citrate, esculin
Fluka chemika-Switzerland	H_2O_2, Glucose, dextran, 99% alcohol. Carbazole ,sucrose ,raffinose, bendict solution, Gram stian kit
B- Culture media	
Hi medium	Blood agar base, MacConkey agar , Agar-agar, Muller-Hinton agar, Nutrient agar, Nutrient broth

2-2-3 Diagnostic Disks:-

Vancomycin (30 μg/ml) (Himedia, India)

2-3 Methods:-

2-3-1 Collection of Specimens:-

The proper specimens collected for bacteriological analysis are described below. Those specimens were collected in proper way to avoid any possible contamination. The sample were generally collected from patients with dental caries and periodontal disease .

The swabs were inserted into the middle of cavity in case of carious tooth, while swabs were taken from dental plaque in case of periodontitis and gingivitis .

Swabs for culture were placed in tubes containing normal saline to maintain the swab moist until being taken to the laboratory. Each specimen was immediately inoculated on blood agar plates , nutrients agar , chocolate agar and MacConkays plates. All palates were incubated aerobically and anaerobically by candle jar at 37 C° for 24-48 hrs.

2-3-2 Preparation of the Reagents and Solutions:-

2-3-2-1 Oxidase Reagent:-

This reagent was prepared by dissolving 1 gm of (tetramethyl-paraphenylene-diamine-dihydrochloride) in 100 ml of D.W and immediately used for identification of the bacteria (Baron, *et al.*, 1994).

2-3-2-2 Catalase Reagent :-

This reagent was prepared in (3%) solution using H_2O_2 dilute by D.W and stored in a dark container and used for identification the of bacteria (Baron, *et al.*, 1994).

2-3-2-3.Phenol Red Reagent

It was prepared by dissolving 0.1 gm from phenol red dye in 300ml of ethyl alcohol (95 %) then the volume was completed to 500 ml by D.W. It was used to detect the acidity of the media. (Baron, *et al.*, 1994).

2-3-2-4- McFarland Tube Standard (0.5) :

A barium sulfate turbidity standard solution equivalent to a 0.5 McFarland standard was prepared as described by NCCLS (2003a),as follows :

- A 0.5- ml aliquot of 0.048 M $BaCl_2$ (1.175 %w/v $BaCl_2.2H_2O$) was added to 99.5 ml of 0.18 M H_2SO_4 (1 % v/v) with constant stirring to maintain a suspension.
- Correct density of the turbidity standard was verified by using reading the absorbance at 625 nm . the absorbance should be 0.08 to 0. 10 for the McFarland standard.
- Barium sulfate suspension was distributed in 4 ml aliquots in to screw-cap tubes, which were tightly sealed and stored in the dark at room temperature.

- Barium sulfate turbidity standard was vigorously agitated on a mechanical vortex mixer before each use and inspected for uniformly turbid appearance.
- Barium sulfate standard should be replaced or their densities verified monthly.

2-3-2-5- Normal Saline:

This solution was prepared by dissolving 0.85 gm of sodium chloride BDH in small amount of D.W and completing the volume to 100 ml (0.85 %) ; then sterilized by autoclave 121 C for 15 minute.(Garrvy,*et al.,*1979) . Used for preparation of formal saline

2-3-2-6 Formal Saline:

This solution was prepared by adding 0.05 ml. from formaldehyde (H-CHO) BDH company (M.Wt. 30.3) to 99.5ml (v/v) from 0.85% normal saline to produce 0.05% concentration ; it was used to dissolve the salivary immunoglobulin (Lehmam *et al .,* 1998)

2.3.2.7. Acetate Buffer Solution

This buffer is prepared by dissolving 8.2 gm of sodium acetate in 300 μL glacial acetic acid, then the final volume is compeleted to 1L with D.W . The pH is 5.8, then the solution is sterilized in autoclave .(Baron ,*et al.,*1994). The pH was readjusted after outoclave.

2.3.2.8. Tris Buffer Solution

This buffer was prepared by dissolving 12gm from the tris base (NH2C(CH3OH))3 TAAB company (M. Wt 121.4) in small amount of D.W. and completed to 1 liter. The pH was adjusted to 7 by adding HCL (o.1 N) . This solution was used to prepare poly ethylene glycol solution (Johnston and Thorpe,1982) .

2-3-2-9 Poly Ethylene Glycol Solution (PEG)

The required concentration of PEG solution was 6 %. It was prepared by dissolving 6gm from PEG (HO(C2H4O)nH) BDH company (M.Wt. 249.5) in a small amount of Tris buffer and complete the final volume to 100ml D.W. This solution is used to separate immunoglobulin from saliva , serum and urine (Shnawa and Mehdi , 2004 ; Johnston and Thorpe, 1982).

2-3-2-10 Biuret Solution

This solution was prepared by dissolving 3gm from cupper sulfate (CuSO4 . 5H2O). BDH company (M.Wt 166) in half letter of D. W. with adding 9gm from sodium potassium tartarate (NaKC4H4O6 . 4H2O-(BDH company) (M. Wt. 249.5). After dissolving those three components , 100ml from sodium hydroxide (NaOH 0.6) was added and the final volume was completed to 1litter by adding D.W.

This solution was used in biuret method to measure the concentration of immunoglobulin (Ross , 1985)

2-3-2-11 Standard Albumin Solution

To prepared standerd albumin solution 15 gm from dry-egg albumin-BDH company (M. wt 65400) was dissolved in a small of sodium hydroxide 0.6 N and the final volume completed to 1 liter by Na OH(0.6 N).

The final concentration of albumin was 15 gm/liter. Standard dilutions from this solution were prepared (1 :1 , 1:2 ,1 : 4 , 1 : 8 , 1 : 16 ,1 : 32 ,1 : 64) that represent the following concentrations 7.5 ;3.74 ;1.8 ; 0.927 ;0.437 ; 0.0224 ; 0.0112) gm/L respectively .These dilutions were used to prepare standard curve to detect the concentration of immunoglobulin (Ross ,1985).

2-3-3 Preparation of Culture Media:-

The general culture media described below were prepared according to the methods mentioned for each media and used in appropriate experiments:-

2-3-3-1 Blood Agar Medium:

Blood agar medium has been prepared according to MacFaddin, (2000) by dissolving 40 gm blood agar base in 1000 ml D.W. and autoclaved at 121Cº for 15 min, then cooled to 50 Cº and 5% of human blood was added. This medium was used to cultivate bacterial isolates and to determine their ability to lyse the blood.

2-3-3-2 Chocolate Agar Medium:

Chocolate agar medium has been prepared by dissolving 40 gm of blood agar base in 1000 ml D.W. and sterilized by autoclaving. Then 8% of human blood was added to the medium after cooling to 80 Cº. This medium was especially used for isolation and cultivation of bacteria that need 5-10% CO2 tension (Baron, *et al.*, 1994).

2-3-3-3MacConkey Agar Medium:

MacConkey agar medium has been prepared according to the method recommended by the manufacturing company and it is used for the primary isolation of most G-ve bacteria and differentiation of lactose fermentative from the non lactose fermentative (Collee *et al.*, 1996).

2-3-3-4 Nutrient Agar Medium:

Nutrient agar medium has been prepared according to the manufacturing company. It has been used for general experiment isolate culture, cultivation and activation of bacterial isolates when it is necessary (Macfaddin, 2000).

2-3-3-5 Mannitol Salt Agar Media:

This media has been used as a selective media for the isolation of Staphylococci and differentiation of ***S. aureus*** (Macfaddin, 2000)**.**

2-3-3-6 Muller- Hinton Agar:

Muller- Hinton agar has been prepared according to the method recommended by (Vall, *et al.*,1999) and it is used in anti-microbial susceptibility testing

2-3-3-7 Sugar Fermentation Media :-

Medium is composed of :-

A. Basal medium :-

It was prepared by dissolving 10 ml peptone , 1gm meat extract (Difco) ,5gm sodium chloride (NaCl) and 0.08 gm phenol red (BDH) in one liter D.W. ,then the pH was adjusted to 7.4 . The medium was distributed in test tubes and Durham tubes were added to each test tube then sterilized by an autoclave at 121 C for15 min. (MacFaddin,2000).

B.Sugar Solutions :-

Sugar solution was prepared by dissolving 1gm sugar in 1000 ml D.W. and sterilized by filtration with milipore filter paper ,then 0.1 ml sugar solution was added to each test tube

(Item -1) containing 5 ml from basal medium . the medium was used to diagnosis the bacteria that have the ability of sugar fermentation . (MacFaddin,2000).

2-3-3-8 M9 Media:

Six gm of Na_2HPO_4, 3 gm of KH_2PO_4, 0.5 gm of NaCl, and 1 gm of NH_4Cl; were dissolved in 950 ml of D.W. with 2% agar, and then sterilized by autoclave. After cooling, 2 ml of 1M of $MgSO_4$, 10 ml of 20% glucose and 0.1 ml of 1M of $CaCl_2$ (sterilized separately by filtration) were added. Then the volume was completed to 1000 ml. This media was used for the detection of the Siderophores and Extracellular Proteases production (Miniatis, *et al.*, 1982).

2-3-3-9 Esculin Media:

The esculin was 6,7-dihydroxy coumarin 6-glucoside which had inhibitory effect on xanthin oxidase enzyme(Holt,*et al.*,1994). This media was made by preparation of nutrient agar supplemented with 0.4gm ferric ammonium citrate and 4 gm esculin.

2-3-3-10 Selective Media

This media was prepared by preparation of nutrient agar supplemented by sodium azide (1 :16000) to inhibit G –ve and crystal violet (1:500000) to inhibit staphylococci. This media providing advantages of enrichment and selection by providing optimal conditions for streptococcal growth while inhibit other organisms (Marie, *et al.*,1985).

2-3-3-11 Motility Test Medium (Semisolid Media):-

It was used to detect the motility of microorganisms. It was prepared according to the method suggested by Macfaddin(2000) by added 4gm of agar-agar to the 100ml of neutrient broth, sterilized by outoclaving and dispensed in tubes of 5 ml.

2-3-4 Laboratory Diagnosis:

According to the diagnostic procedures recommended by Maccfaddin (2000); (Holt, *et al.*, 1994); (Collee, *et al.*, 1996); (Baron, *et al.*, 1994).,the isolation and identification of G+ve and G-ve bacteria in patients with dental diseases were performed as follows:-

2-3-4-1 Microscopical Examination and Colonial Morphology:

A single colony was taken from each primary positive culture and its identification depended on the morphology properties (Colony size, shape, color and natural of pigments, translucency, edge, and elevation, and texture), and then colonies suspected to be pathogens were selected and investigated by gram-stain to observe a specific shape, color, aggregation and specific intracellular compounds. After staining the bacteria by gram stains, specific biochemical tests were done to reach the final identification.

2-3-4-2 Physiological and Biochemical Tests

2-3-4-2-1 Oxidase Test:

A filter paper circle was placed into a sterile plastic disposable petridish and moistured with several drops of the freshly prepared oxidase reagent, then a small portion of the colony to be tested was removed and rubbed on the filter paper. Changing in the color to blue or purple within 10 seconds indicated for a positive result (Baron, *et al.*, 1994

2-3-4-2-2 Catalase Test:-

By streaking the nutrient agar medium with the selected bacterial colonies and incubated at 37 Cº for 24 hrs then transfer the growth by the steak and put it on the surface of a clean slide and add a drop of (3% H_2O_2). Positive result when the gas bubbles appear (Baron, *et al.*,1994).

2-3-4-2-3 Esculin hydrolysis test :

Esculin medium was inoculated and incubated at 37 Cº for 24 hrs. Changing the colour of medium and colonies to black indicates esculin hydrolysis.

2-3-4 -2-4 Coagulase Test:

This enzyme was tested by two methods:-

a.Slide test for bound coagulase (clumping factor): A drop of human plasma was placed on a clean, dry glass slide, a drop of D.W. was placed next to the drop of plasma as a control. By a sterile loop an amount of the isolated colony was emulsified with each drop. When clumping the plasma, bacteria was observed and a smooth homogenous in the control; the result was recorded positively (Baron, *et al.*, 1994).

b. Tube test for free coagulase:

half ml of Human plasma was placed in a glass tube and a visible portion of growth from isolated colonies was emulsified in the plasma by rubbing the material on the side of the tube while holding the tube at an angle. then the suspension was incubated for 1-4 hrs at 37 Cº. The presence of clot that cannot be resuspended by gentile; shaking was recorded as a positive result. The organism that fails to clot the plasma within 24 hrs is considered as coagulase negative (Baron, *et al.*, 1994).

2-3-4-2-5 Mannitol Salt Agar Test:-

The differentiation between ***S. aureus*** and other Staphylococci e.g. ***S. epidermidis*** was done by sub culturing selected colonies on mannitol salt agar for 24 hrs at 37 Cº. Colonies surrounded by a

yellow halo indicating mannitol fermentation and isolates colony, is ***S. aureus*** (MacFaddin, 2000).

2-3-4-2-6 Vancomycin Susceptibility Test:-

A half of 8% human blood agar plate was streaked with an inoculum form a pure isolates of the organism to be tested. Then an Vancomycin disc was placed in the center of the inoculum and incubated for 24 hrs at 37 Cº in a candle jar. Then observation of zones of growth inhibition greater than 14 mm surrounding the disc was considered positive and it was presumptive indication of ***St.mutans,*** (Baron, *et al.*, 1994).

2-3-4-2-7 Motility Test

This test was done by inoculated the tube that contained semisolid media with tested bacteria by stabbing method and incubate at 37 Cº for 24-48 hr. The disseminating of growth outer the stabbing line indication for positive result (MacFaddin ,2000).

2-3-5 Virulence Factors Tests:

2-3-5-1 Blood Hemolysis Production Test:

Blood agar medium was streaked with a pure culture of bacterial isolate to be tested and incubated at 37 Cº for 24-48 hrs. The appearance of a clear zone surrounding the colony is an indicator of β- hemolysis while the greenish zone is an indicator of α- hemolysis (Cowan, 1985).

2-3-5-2 Extracellular Protease Production Test:

This method was carried out by using M9 media supplemented with 2% agar. After sterilization in autoclave and cooling at 50C^{o}, 0.25 mg/L glucose (sterilized by filtration) was added. Then, the media was supported by 1% gelatin. After the inoculation of this media with bacterial strain and incubation for (24 - 48) hours at 37C^{o}, 3 ml of Trichloroacetic acid (5%) was added to precipitate the protein. The positive result was read by observing a transparent area around the colony (Piret , *et al.*, 1983).

2-3-6 Detection of Dextranase Production

Two procedures were used for isolation of dextranase producing *St.mutans* from oral samples .

A. This method was carried out using M9 media supplemented with 2% agar.After sterilization by autoclave and cooled at 50 C°, sucrose 10 % and dextran 1% (sterilized by filtration) were added to this media ,and when this media became solid , holes were made and filled by broth media contain *St. mutans* then incubated for 48 hrs at 37 C°. Three ml of benedict solution were added and incubated at 37 C°for (20-30 minute). Positive results were read by observing a purple –brown discoloration around the colony. (Dr. Mohammed Sabri,Babylon Univ.,Personal communications).

B. The second procedure was done by using broth of M9 supplemented by 10 % sucrose then cariogenic *St.mutans* inoculated

and incubated for about 48 hrs. Benedict solution (3 ml) were added to this broth and heated in water bath at (100 C) for about 20 to30 minute. The change in color from blue to brown or purple also indicated positive result (Hamada ,*et al.*,1975) and (Simonson ,*et al.*,1994)

2-3-6-1 Effect of Some Antibiotics on Dextranase Production

M9 agar medium was prepered, by dissolving Six gm of Na_2HPO_4, 3 gm of KH_2PO_4, 0.5 gm of NaCl and1 gm of NH_4Cl; were dissolved in 950 ml of D.W. with 2% agar, and then sterilized by autoclave. After cooling, 2 ml of 1M of $MgSO_4$, 10 ml of 20% glucose,1ml of 1% dextran and 0.1 ml of 1M of $CaCl_2$ (sterilized separately by filtration) were added, then the volume was completed to 1000 ml. afterwards, the antibiotics solution at concentration 0.1mg/ml sterilized by filtration was added to the medium. The same medium was prepared but without antibiotics which represents the control medium. The media were poured in sterilized petridishes and left to solidify. By making hole in these media , and filling these holes with bacterial broth of *St. mutans*. Then these plates were incubated at 37C°for 24-48hr. Three ml of benedict solution were added and incubated at 37 C°for (20-30 minute). The effect was read by disappering the discolaration arround these holes. (Dr. Mohammed Sabri,Babylon Univ.,Personal communications).

2-3-6-2 Effect of Different Sucrose Dilutions on Dextranase Production

M9 media as perppared above, to which sucrose dilutions at concentration from (0.1-1) mg/ml sterilized by filtration were added to this medium.The same medium was prepared but without sucrose solution which represent the control medium. The media were poured in sterilized petridishes and left to soulidify, by making hole in these media , and filling these holes with bacterial broth of *St. mutans*. Then these plates were incubated at 37C°for 24-48hr. The effect was read by disappering the discolaration arround these holes. as suggested by my supervisor(Dr. Mohammed Sabri, Babylon Univ.,Personal communications).

2-3-7 Antimicrobial Susceptibility Tests :-

It was performed according to Kirby-Bauer,*et al.,* (1966). Method by using a pure culture of previously identified bacterial organism. The inoculum to be used in this test was prepared by adding growth from 5 isolated colonies grown on blood agar plate to 5 ml of Nutrient broth. This culture was then incubated at37 C for 2 hours to produce standardized bacterial suspension of moderate turbidity.

I. A sterile swab was used to obtain an inoculum from this culture, That was streaked on a Muller-Hinton plate.

II.The antibiotic discs were placed on the surface of the medium at evenly spaced intervals with flamed forceps or a disc applicator

III.Incubation was usually overnight with an optimal time of 14 hours at 37Cº. Antibiotic inhibition zones were measured using a caliper. Zone size size diameters were interpreted according to that recommended by clinical laboratory standards institue documentations (CLSI,2007). Antibiotic disc is prepared by Oxiod Company with the following disc potency :-

No	Antibiotic*	Disc-potency µg/ml
1	**AMP**	**10**
2	**AMX**	**10**
3	**AMC**	**20/10**
4	**APX**	**15**
5	**CTX**	**30**
6	**CIP**	5
7	**TR**	**30**
8	**DO**	**30**
9	**ER**	**15**
10	**STX**	**30**

* AMP: Apmpicilln ,AMX :Amoxillin **,**AMC :Amoxiclave **,** APX: Ampiclox , CTX:Cefotaxime,CIP:Ciprofloxacin**,**TR :Tetracycline,DO :Doxycyclin, ER :Erythromycin**,** SXT:Trimetheprime- sulfamethoxazole.

2-3-8- Measurement of Salivary Immunoglobulin Concentration.

2-3-8 -1 Separation of Immunoglobulin from Saliva

The material used for separation of Immunoglobulin from saliva was polyethylene glycol (M.Wt.,6000) (Johnston and Thorpe, 1982 ; Alsaadi,1998; Shnawa andAl-amidi 2005) .

1. Ten mls from saliva sample was centrifuged at 3500 rpm for 5 minutes , with removing the precipitate and collecting supernatant.
2. Eight ml from supernatant were filtered by using moisture filter paper.
3. five ml of (PEG) were added to 5 mls of filterated saliva and kept in refrigerator for one hours.
4. The mixture was centrifuged in 6000 rpm for 30 minutes.After that the precipitate was dissolved in 0.5 ml of formal saline and transferd to an Appendrof tube.

2-3-8-2Measurement of Salivary Immunoglobulin concentration.

The concentration of sIg-A in saliva ,was measured by Biuret method (Bienenstock and Tomasi,1968).

1. 5 mls of Biuret solution was tubed in spectronic tube .
2. Immunoglobulin solution (0.2ml) was added to the Biuret solution . This tube was represent as tube of test run
3. D.W. (0.2 ml) was added to 5mls of Biuret solution . this tube is considered as control tube

4. The tubes were mixed and left aside for 30 minutes in room temperature

5. The optical density was measured on wave length 450nm. The immunoglobulin concentration was measured depending on the standard curve that has been prepare from dilutes of egg albumin solution .(Figure 2-1).

2-3-9- Statistical Analysis:

1- Differences between males and females were compared by X^2 tests under confidence level of 0.95; the ***P*** value ≥ 0.05; was considered a non significant difference

2-differences between smokers and non smokers , diabetic and non diabetic patients were compared by X^2 tests under confidence level of 0.95 ; the *P* was less than 0.05 .

3-The equation that was used to calculated the concentration of immunoglobulin was depending on the equation of simple linear regression .This equation was as the following :

$\hat{Y} = a + b\ Xi$

$\hat{Y} = -0.31 + 93.41Xi$

$\hat{Y}$ = concentration of immunoglobulin

Xi=optical density

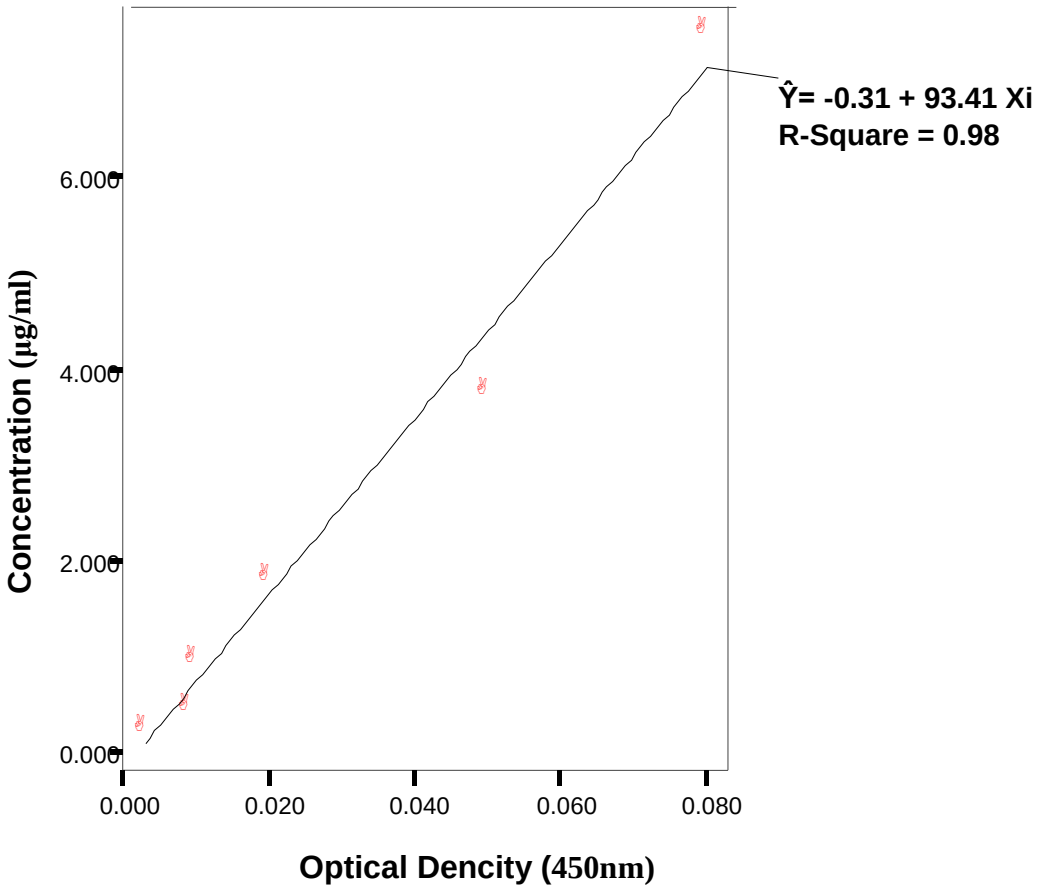

Figure (2-1) Standard curve between standard albumin solutions and optical density .

Results and Discussion

3.1. Distribution of patients with dental caries according to sex and age

In this study, the incidence of dental caries among male patients were 26/50(52 %) ,while the female cases were 24/50 (48 %). Table (3-1).The results had indicated that the susceptibility of males and females to infection of dental caries was nearly similar; there was no significant difference , ($P \geq 0.05$), these findings were in agreement with that of Anderson ,*et al*.,(2006), who reported that there was no significant diffrence in the incidence of the dental caries in relation to sex . However ,Dababneh, *et al., (*2007) ,who found that there was significant difference in cases of dental caries where this disease was higher in females than males .

As shown in (table 3-1), it was found that the incidence of this disease was high in age group range from (15 -25) year old ,followed by age group (36-45) year old and the rest age groups had less frequency than mentioned above,these results were in agreement with the results reported by Brattall ,(2000) and Neville,*et al .,(*2002) , who noticed that the prevalence of dental caries increased during childhood and teenagers groups. The results revealed that highest prevalence of dental caries were among the age group (15-25) may be attributed to the frequent consumption of fermentable carbohydrates especially sucrose sugar and by presence of a

cariogenic bacteria (*St.mutans*). A destructive acid produced by this bacteria will dissolve the enamel and dentin of tooth under low pH of saliva . (Peterson ,*et al.,* 2002; Todar , 2008) .

Table (3-1) The distribution of patients with dental caries according to sex and age

Age group	Sex		Total Number	%
	Male	Female		
15-25	8	11	19	38
26-35	3	5	8	16
36-45	7	4	11	22
46-55	4	4	8	16
56≥65	4	0	4	8
Total number	26	24	50	100
Total %	52%	48%	100%	

P ≤ 0.5

3.2 Distributions of patients with periodontal diseases according to sex and age.

The distribution of patients with periodontal diseases according to sex and age was also analyzed ,(table (3-2). It was observed that the prevalence was higher in females than males patients (60 % and 40 %) respectively . These results had indicated that the susceptibility of females to infection with this disease were significantly higher than males, ($P \leq 0.05$) .This finding was compatible with Loesche

,(2000), who had reported that the prevalence of periodontal disease higher in female patients than males ,which may be attributed to changes in female hormones during pregnancy and menstruation periods . In these two physiological conditions the level of progesterone increased ,which leads to vasodilatation .the latter may causes inflammation of periodontal tissues and causes block repair of collagen which is structural protein support the periodontium .(Wie, *et al.*,1986) .Thus ,during these two periods the female patient subjected to sever case of gingivitis and periodentitis .(Waerhaug ,1987),who had reported that during menopuse the periodontal problem will increase due to the loss of estrogen which is very important in maintainance of bone integreity,so its loss lead to osteoprosis and alveolar bone resorption so these lead to periodontal problem . Stamm,(1998), who had also reported that the incidence of gingivitis and periodontitis was markedly increased from 70 % to 90 % during pregnancy . They explained the case as due to increase in the level of pogestron which lead to increase in vascularity of periodontium and it also during pregnancy there is a marked increase in the number of blak-pigmented bacteria which causes periodentitits

It was found that the incidence was high in age groups ranging from (46-55) years followed by those above 60 years old . The rest age groups had less frequency than the mentioned above . These results were in agreement with the results obtained by (Piwowar ,*et al* .,2000), who showed that the risk of periodontal diseases increase with age , and this was significantly at risk in elderly patients who

became more susceptible to infection as result of marked decrease of host defense with the age . This finding was also in agreement with that reported by Shanley and Ahern,(1999) and Loesche,(2000).

Table(3-2) Distributions of patients with periodontal diseases according to sex and ages

Age group	Sex		No.	%
	Male	Female		
15-25	2	4	6	15 %
26-35	3	4	7	17.5
36-45	2	2	4	10 %
46-55	5	9	14	35 %
56 ≥ 65	4	5	9	22.5 %
Total No.	16	24	40	100 %

**** P ≤ 0.5***

3.3 Predisposing factors associated with dental disease :

3.3.1 Diabetes Mellitus

In this study the systemic disorders such as diabetes mellitus among the patient with dental diseases were studied .

The result indicated that dental diseases were frequently occurred in patients with D.M. and these were reported in 32.3 % of cases of dental diseases as shown in table (3-3) .It was also seen that effect f

D.M.among the patients with periodontal disease was significantly higher than those with dental caries .($P \leq 0.05$) 65 % and 35 % respectively . These results nearly in compatible with , Stewart, *et al* ., (2001) ,who found that there was good evidence to support an association between poorly controlled diabetic patients and periodontal disease especialy in long standing and severe cases , This finding was not compatible with that reported by Lindhe, (1996) ,who explained that there is no relationship between diabetic state and dental disease .

West.*et al* .,(1997) ; Piwowar ,*et al.*,(2000) , had mentioned that the possible reasons for increased susceptibility of diabetic subjects to dental infection , could include ,firstly impaired host defense to infection ,secondly excessive release of pro inflammatory cytokine and tissue degrading enzyme

Table (3-3) Distribution of patients with dental diseases according to diabetic state

Diabetic state	Sex		Type of lesion		No.	%
	Male	Female	Dental caries	Dental plague		
diabetic case	18	11	10	19	29	32.3 %
non diabetic case	29	32	40	21	61	67.7%
Total No.	47	43	50	40	90	100 %
Total percetage	52.2	47.8	100%			

* ***P ≤ 0.5***

3.3.2. Smoking

The effect of smoking on development of dental diseases was studied and it was found that 64.2 % of patients with dental diseases had smoking habit as shown in table (3-4) . The results revealed that the effect of smoking among the patients with periodontal disease was significantly higher than those with dental caries ($P \leq 0.05$). These results may explain the direct effect of smoking in predisposing to the change in dental plaque ; pocket flora and its effect in producing vasoconstriction in blood vessels in periodontium , which may lead to reduce availability of serum-derived protective factors such as antibodies and polymorphnuclear leukocytes in periodontal tissue (Zambon,*et al.*,1996; Ryder.,1996 and Tangada,*et al*.,1997).

This finding was indcated that smoking was directly related to prevalence and incidence of periodontal diseases .Furthermore a recent study have shown that the smoking may still significant risk factor in development of dental disease ,due to its effect on local and systemic host respone ,that lead to impair the host respone against infection (Totti ,*et al*.,1994).Another recent study by(Brogestro¨m,*et al* .,2000) suggested that the periodontal disease was significantly higher in smoker patient than non smoker, this was due to that the effect of smoking on healing process after infection because during smoking the nicotine stores in fibroblast cells and impairs their function.

Table (3-4) Distribution of patients with dental diseases according to the smoking state

Smoking case	No.of cases	Type of lesion		Total %
		Dental caries	dental plaque	
Smokers	27	9	18	64.2 %
Non smokers	15	6	9	35.8 %
Total	42	15	27	100 %

* $P \leq 0.5$

3.4 Bacterial Isolates

The results revealed that (47) samples of dental caries gave positive bacterial culture .The other three samples out of (50) swabs show no bacterial growth even after 48 hrs . These three cultures gave fungus growth such as candida. The results of samples collected from dental plague gave (39) positive bacterial culture and only one sample showed no bacterial growth .

This study showed that the most isolated samples from patients with dental diseases gave mixed culture .This may be attributed to the fact that the oral cavity is open growth system , provide favorable environment for colonization of more than 400 spp. of aerobic and anaerobic bacteria ,most of which act as normal flora of mouth (Todar ,2008 and Eley and Manson ,2004) .

A total of 102 bacterial isolates were obtained from the 86 samples of bacterial isolates . From results it was shown that *G+ve* bacteria constituted 80 % (82 of 102) of total isolates and they considered predominant bacteria in dental infections compared to *G –ve* bacteria which constituted about 20 % (20 of 102) .

3.4.1 Bacteriological Aspect of Dental Diseases

In this study out of 102 isolates, only 24 isolates of *St. mutans* were isolated .Fourteen isolates were isolated from dental caries lesions ,and 10 isolates were taken from dental plaque . No isolate of

St. mutans were isolated from the healthy control .This explains that *St. mutans* represent the main causative agent of dental diseases , especially dental caries and to a lesser degree the periodontal disease . Table (3-5) .

These results were similar to those obtained by (Yoo ,*et al* ., (2007) ;Shimotoydome,*et al.* .,(2007) and Todar ,(2008) .

Seventy cases of dental diseases were caused by mixed bacterial types. *St. mutans* (24/102) isolates were most frequent. Followed by ,*Nisseria spp.* and *S. aureus* (15/102) . The aerobes and facultative anaerobic bacteria were found in high frequency (98.2 %) compared to anaerobes which include *Peptostrep*.spp. and *Veillonella* spp.(1.96 %) . Results also showed that *St. mutans* isolated from dental caries(25 %) was more than those isolated from dental plaque (21.2%).

Table (3-5) Distribution of bacterial isolate according to type of lesion

Bacteria	Type of isolate		Type of lesion		Total No.	%
	Single	Mixed	Carious lesion	Dental plague		
St. mutans	9	15	14	10	24	23.5
Nisseria spp.	6	9	8	7	15	14.7
S. aureus	5	10	6	9	15	14.7
Diphtheriod	3	6	5	4	9	8.8
Lactobacilli	4	3	4	3	7	6.8
S. epidermidis	1	5	4	2	6	5.8
Actinomyces	1	5	3	3	6	5.8
St. salivarius	0	5	3	2	5	4.9
St.pyogenes	2	2	1	3	4	3.9
other strep.	0	3	1	2	3	2.9
G –ve rod	1	1	1	1	2	1.9
G +ve rod	0	2	2	0	2	1.9
Peptostrep.	0	2	0	2	2	1.9
G-ve anaerobe cocci(Veillonella)	0	1	0	1	1	0.9
Total	32	70	55	47	102	100 %

These results were attributed to that *St. mutans* bacteria play direct and important role in pathogenesis of dental caries as recorded by other studies such as Sonic and Stephan ,(2003) andTodar, (2008).

This finding was also compatible with results abtained by Falklers,(1998) and Kolenbrander,(1998), who reported that *St. mutans* was the principle causative agent for tooth decay and also play an important role for growth of other types of microorganism by production offavorable environment through accumulation of bacterial plaque on tooth surface ,partly due to *St. mutans* its self ,which is capable of manfactucturing the dextran bulk of the plaque by its glycosyltransferase enzyme .

Nisseria spp. was the second type of bacteria isolated from dental carious lesion and dental plague (14.7 %). This result was expected for this organism due to many reasons. Firstly ,Nisseria is the most prominent microflora isolated from oral cavity as dental plaque flora . Secondly,it is isolated from carious lesion and act as commensal bacteria , having no role in pathogenesis of dental caries but it enters the oral cavity and respiratory tract as normal flora .This result was also reported by (,Kolenbrander and London ,1993; Sanai , *et al*.,2003).

Other bacteria that had been isolated was S.aureus which represent (14.7 %) .This frequency may be due to , firstly , it may enter the

oral cavity from exogenous source as normal flora.Secondly , *S. aureus* may inherent nature of developing resistant strains for antibiotics , *S. aureus* also contain teichoic acid ; lipotechoic acid and capsular matrial which facilitate the adherence of these bacteria to oral epithelium. This agree with result mentioned by (Brook ,1998) .

Lactobacilli and *Actinomyces spp*. which constitute (6.8 % and 5.8 %) respectively as shown in table (3-5) were considered another causative agents for dental caries and to lesser extend periodontal disease in second degree after *St. mutans* . Sanai ,*et al* .,(2003)and Todar ,(2008) showed that dental caries pathogens included Lactobacilli and *Actinomyces* spp., responsible for progression of lesion ,were isolated from deep layers of carious cavity which indicated that these two bacteria also had an important role in pathogensis of dental caries .

S. epidemidis and Diptheriod spp. were found in (8.8 % and 5.8 %) respectively. Those microorganism were previously regarded as non pathogenic and contaminant from skin , or as micro flora of the mouth .However they may have a role as apportunistic pathogens in developing dental infections in immunocompromized patients (Johnson ,*et al.,* 1990) .

St. pyogenes (3.9 %) was also isolated from oral cavity but in low percentage.This bacteria was also expected in oral cavity which come from tonsils and nasopharangial region ,and considered as the

main pathogen causing tonsillitis and pharangitis (Brooks ,*et al* ,2007) .

St. salivarius constituted (4.9 %) from total bacteria isolated from dental caries and dental plaque . Eely and Manson ,(2004), had mentioned that *St. salivarius* regarded the second pathogen that follow *St. mutans* in causing dental infection . Also *St. salivarius* were isolated from oral epithelium ,including the surface of toungue ;check and buccal and lingual mucosa of oral cavity (Todar ,2008) .

Other types of bacteria isolated from oral cavity were like *G +ve* and *G –ve* areobic rods which isolated in low frequency ,constituted 1.9 % . These also were expected to colonize the oral cavity ,because the later was considered the portal of entry for bacteria including numerous microorganism ,into alimentary and respiratory systems. Normally these bacteria do not cause disease and are washed away with swallowed saliva into distal parts of the alimentary tract .(Johuns ,*et al.,* 1998 and Scully ,1999) .

Finally ,anaerobic bacteria were also isolated in low frequency such as *Peptostrep. Spp.* which is *G +ve* anaerobe cocci and *Veillonella spp*. which is *G –ve* anaerobe cocci .They isolated only from dental plaque and not isolated from any cases of dental caries . These two pathogen were associated with progressive periodontitis and did not play any role in development of dental caries .This result was in agreement with result recorded by Hughes ,*et al.*,(1988) ; Donkers loot and McCabe(2004) ,who had isolated these two

anaerobe bacteria from surface of tongue and subgingival dental plaque as putative pathogen causing periodontitis .

The mixed bacterial types (70 cases) were related to two different genus and species .As shown in table (3-6) , this may describe symbiosis interaction between these different species in oral cavity . *St. mutans* were (14) isolates which peredominant in mixed infections This result was also recorded by Liligemark and Bloomquest,(2002) ; Todar,(2008),who showed that *St.mutans* play important role in plaque formation due to its ability for production of extra cellular poly saccharide (dextran) matrix of dental plaque ,that provid, a suitable invironment for growth of other types of microorganism. On the other hand it have ability for production extra cellular protease which is effective against IgA so it support growth of other bacteria . (Cox, *et al.*,1993).

The mixed growth in oral cavity attributed to presence of dental plaque biofilm ,which is community of microorganisms. This ecosystem have dynamic balance between enterance of bacteria and host defense aimed to their removal .It also increases the resistance to antimicrobial agent by block its enterance to bacteria inside the plaque.(Hughes,*et al.*,1988).

Other mixed growth was between *St. mutans* and Lactobacilli and represented by two cases as shown in table (3-6) .This finding agrees with Todar,(2008). was indicated that This result was attributed to that Lactobacilli are aciduric so they preferable low pH produced by

fermentation of cariogenic carbohydrate (sucrose in diet) . This low pH will lead to develope cavitation lesion that support growth of lactobacilli .

Anaerobic bacteria were mostly isolated in mixed culture with facultative anaerobic bacteria (*St.pyogenes*) and represented in one case as shown in table (3-6). This result was in agreement Loesche,(2000) who found that anaerobes represent about 0.9% of all isolates. This pattern of mixed culture between aerobic and anaerobic bacteria may be attributed to the synergistic relationship between aerobes and anaerobes ,in which the aerobic bacteria removes oxygen, produces substances that lower the potential of the tissues, or provide nutrients that are necessary for the proliferation of the anaerobic pathogens, whereas anaerobic bacteria alone does not usuallly establish themselves in a disease process.

Also other types of anaerobic bacteria were recorded in mixed culture were *Peptostrep. spp*. with *Nisseria spp*. in two cases . These results recorded by Moore, (1994).Who indicated That these species present in coaggregation with each other ,which plays critical role in the ecology of bacterial growth in oral cavity.

Table (3-6) Types of bacteria isolated from mixed growth

Mixed growth Bacterial isolates	NO.
St.mutans+ *Staph. Spp.*	9
St. salivrius + *Nisseria* Spp.	3
Staph.Spp.+ *Lactobacilli*	3
St.mutans + *Lactobacilli*	2
St.mutans+ *Nisseria* Spp.	2
Peptostrep. + *Nisseria* Spp.	2
St.mutans +*G –ve rod*	1
Staph.Spp.+ *S.pyogenes*	1
St. salivarius+ *Staph.Spp.*	1
Veilloneilla + *St.pyogenes*	1
*Staph.*Spp.+*Diphtheriod*	1
Diphtheriod +*Other Strep.*	1

3.4.1.1 Effect of Some Antibiotics on *St. mutans*

Some antibiotics are used to show their effect on *St. mutans* isolates . It has been found that most isolates showed few resistance to one or more of these antibiotics.

It has been found that (71.2%) of *St. mutans* isolates were resistant to Erythromycin , while the resistance for Tetracycline and Doxycyclin , were (69.3%) ,whereas some isolates were resistant to STX (Trimethoprim-Sulfamethoxazole) (67.1%).(Figure 3-1).

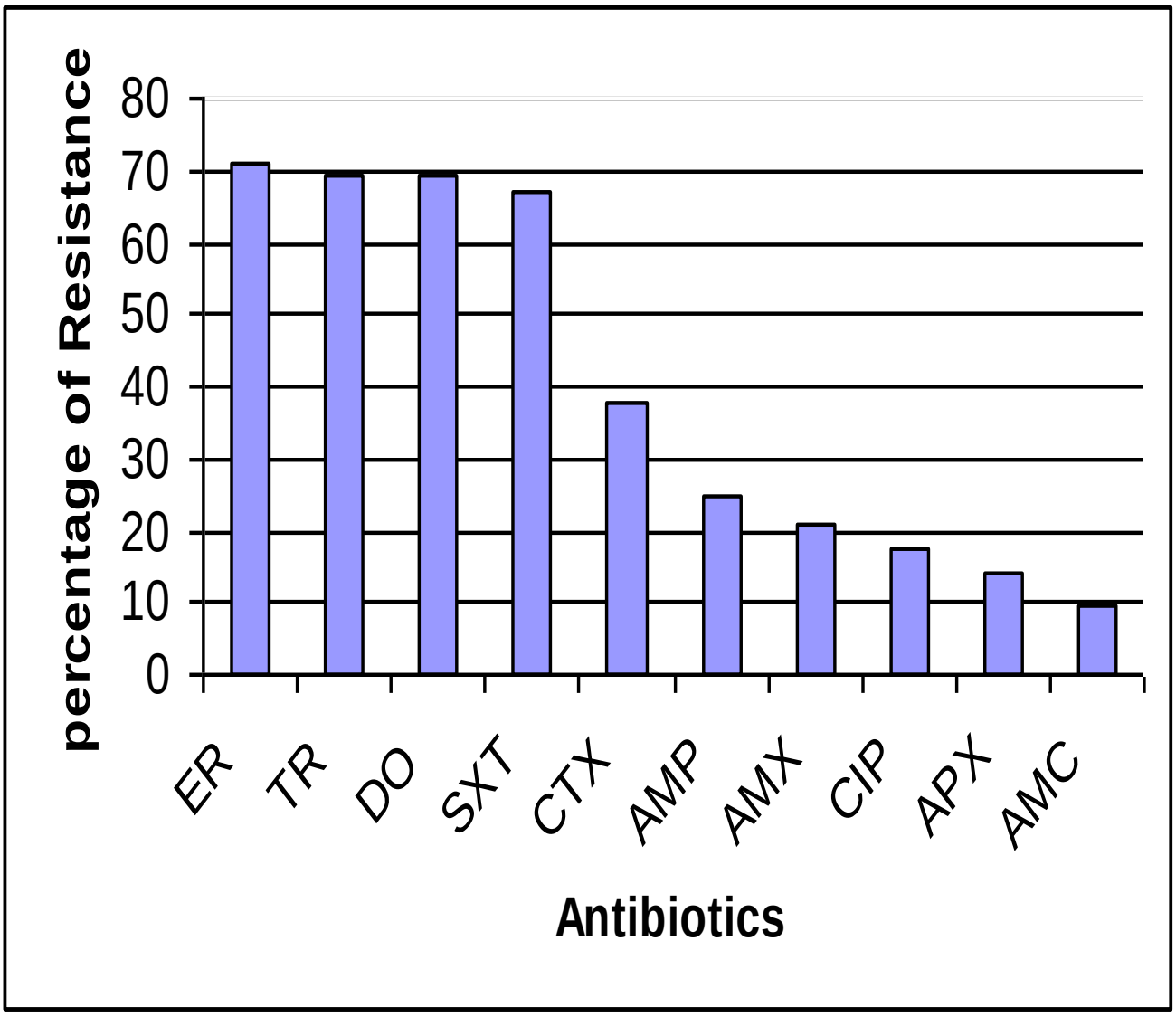

Figure (3-1). Antibiotics resistance of *St. mutans*

In addition, the resistant rate to Cefotaxime was (38%) and the suscepability rat for Ampicillin and Amoxicillin were (75% and 79%) ,respectively.

On the other hand , most of these isolates were susceptible with higher degree to Amoxiclave (90%) and Ampiclox (86%) , while these isolates were susceptible to Ciprofloxacin (83.7%) . Figure (3-1).

The results showed that (71.2%) of *St. mutans* isolates were resistant to Erythromycin .This result was in agreement with the result obtained by DeAzavido ,*et al.,(*1999) ,who had indicated that (75%) of *St. mutans* were resistant to Erythromycin , but this result was in contrast with results of Jarvinen ,*et al.,(*1993) ,who had indicated that 100 %of *St. mutans* were susceptible to Erythromycin . Also Gamboa ,*et al*.,(2000) and Clewell ,(1991) studied macrolid effect on *St. mutans* in children and they had found that 38.5 % of isolates were resistant to Erythromycin .

Erythromycin is one of antibiotics that commonly used in dentistry .The resistance to it may be due to acquisition of one of 21 ert gene ,these code for rRNA methylase that bring about methylation of adenine residue in 23s RNA , preventing the binding of macrolids to 50s ribosomal subunit (Bahal and Nahata ,2000) .

Other mechanisms of resistance by which the bacteria express macrolid resistance include drug inactivation by enzyme and efflux of antibiotics by ATP binding transporter (Leistevuo ,*et al*.,2000).

The results had shown that (69.3) of *St. mutans* isolates were resistant for both Tetracycline and Doxycyclin , these results were similar to results of Murray ,*et al* (2003) who had reported that (64 %) of *St. mutans* isolate were resistant to Tetracycline and Doxycyclin . However , Ferretti and Ward (1998) ,who had found that 50% of *St. mutans* were susceptible to Tetracyclin and Doxycyclin.

Brooks ,*et al*.,(2007) , had mentioned that Tetracycline and Doxycyclin are bacteriostatic agents ,which act against G +ve and G – ve bacteria.They act by attaching to tRNA and by that prevent complexation of aminoacyle tRNA with mRNA of 30s subunit.

Mechanisms by which the bacteria resist to Tetracycline include synthesis efflux pump ,production of ribosomal protection and enzymatic modification of the antibiotic. Tetracycline resistance is encoded tet gene , most of which found in oral species .(Clewell ,1991) . Doxycyclin resistance transport with tetracycline resistance on the same plasmid .(Murray ,*et al.*,2003) .

In oral cavity Tetracycline and Doxycyclin resistance also had been shown after administration of this antibiotic , where the resistance was only seen in 1.7 % of isolate in patients who are not treated with these antibiotics (Fiehn and Wester,1990) ;Olsvik ,*et al.*,1995 ; Killoy ,2002).

Regarding ,the combination of Trimethoprim and Sulfamethoxazole (co –trimoxazole). This result has been shown that about (67.1 %) of *St. mutans* isolates were confer resistance to this antibiotic.This combination act by inhibition the bacterial growth by inhibiting synthesis of dihydrofolic acid .(Lureuce ,*et al.*,(1999).

This result was in agreement with Weld and Sand ham (1999). Who had found that 55 % of *St. mutans* was resistant to this antibiotics.

Another study reported that 70 % of isolates are resistant to co-trimethoxazole.(Teng,*et al.*,1998).

The mechanism of bacterial resistance to TMP/SMX is mediated by modification of the antibiotic target and prevention the access of antibiotic to the target ,by synthesis enzymes in sensitive to action of drug (Goodson, 2000 and Ciancio ,2002) .

As shown in (Figure 3-1), the result showed that about (38%) of isolates were resistant to Cefotaxime. This result was nearly compatible with those pointed by Gums and Gainesville , (2000) ,who found (29.3 %) of isolates were resistant to Cefotaxime .

Cefotaxime is the third generation of Cephalosporin.The resistance of *St. mutans* to Cephalosporin may be due to the synthesis of β-lactamase as well as loss PBPs by mutation which may confer resistance to this antibiotic ,*St. mutans* produce plasmid and/or chromosomal mediated β-lactamases . Other mechanism of resistance to Cefotaxime by active efflux system ,act as wide transporters for whole ring of antibiotic ,(Martinez ,*et al*.,2004) .

Also , the results of the study were showed about (83 %) of isolates were sensitive to ciprofloxacin . Ciprofloxacin , one of quinolons antibiotics that not used permanently in dental practice. This interpreted the low level of resistance to Ciprofloxacin ,(Teng ,*et al.*, 1998) .

Ciprofloxacin ,is a bacteriocidal drug .They affect on G+ve and G –ve. The resistance to fluroquinolone was through chromosomal mutation or alteration affecting the ability of antibiotic to penetrate the bacterial cell walls (Weld and Sandham ,1999) .

Beta lactam antibiotics had also been tested ,they were frequently used in dentistry ,which included (Ampicillin ,Amoxicillin) and new generations of β –lactams (Amoxiclave and Ampiclox) , the resistance rate of *St. mutans* to Ampicillin was (25%) and its resistance to Amoxicillin was (21%) ,while its resistance to Amoxiclve and Ampiclox were (9.5 %and 14%). These results are correlated with those of Povida ,*et al*., (2007),who have shown that most *St. mutans* isolate have low resistance to Ampicillin and Amoxicillin while it was completely sensitive to Amoxiclave and Ampiclox (100 %) .

Recently Amoxiclave and Ampiclox are used in dental practice because of the little resistance of these antibiotic compared with old generations of β –lactam .(Povida,*et al.*,2007)

Gamboa ,*et al.,(*2000) ,who indicated that all isolates of *St. mutans* highly susceptible to Ampicillin as well as Amoxcillin .

On the other hand these results were in disagreement with results of Jarvinen ,*et al* (1993) ,who have indicated that all *St. mutans* isolates (100 %) were susceptible and Amoxicillin .

Also Eley and Manson , (2004) ,how indicated that all isolate are highly susceptible to Amoxiclave (100 %) .

The possible mechanisms for resistance to β –lactams antibiotics are the enzymatic hydrolysis of β –lactam ring by β –lactamase , failure of antibiotics to penetrate to PBPs and low affinity to bind of antibiotic to PBPs also confer resistance to those antibiotics .

The resistance of the bacteria to β –lactam also occur by extended spectrum beta lactamases and this enzymes currently inhibited by clavulanic acid ,(Masters ,1997). Morever , new generation of β –lactam are better to be used in stead of Ampicillin and Amoxicillin like those containing β –lactamase inhibitors namely clavulanic acid (Eley and Manson,2004). The resistance of *St. mutans* to those β –lactam is probably attributed to the fact that most clinical isolates produce different plasmid and /or chromosomal mediated β –lactamase enzyme (Gamboa ,*et al.*,2000) . Also for most oral Strep. ,the resistance attributed to the repeated using of these antibiotic and also to the presence of genes responsible for resistance plasmids or transposons .(Teng, *et al*, 1998) .

3.4.1.2 Protease production by *St. mutans*

In this expermint, 14 *St. mutans* isolates were selected for study the the protease production. The results showed that 5/14 (35.7 %) of *St. mutans* isolates produce protease on M9 media as shown in table (3-7). The results of present study agree with the results of Jackson,*et al.,(*1997) , who had indicated that oral Streptococci (*St. mutans ,St.*

intermedius, St. oralis ,St. salivarius and *St. sanguis)* have the ability to produce extra cellular proteases .

St. mutans can produce extra cellular proteases when they reach the oral mucosa may often encounter the sIgA ,which could inhibit their adherence and attachment to the tooth surface . They are able to evade the action of secretary-Ig A by producing Ig A protease that inactivate IgA , which play central role for local and humeral immune response in oral cavity against dental infection.Thus *St. mutans* have ability to colonize the tooth surface (Gazie ,*et al.*,1997) . A52 –KDa protease of *St. mutans* have been purified and characterized by Simth,*et al.,(*1994).

Protease contribute directly to pathogenesis of dental disease by degrading the host defense protein such as immunoglobulin and complement and/or cleaving other streptococcal surface protein. Also they are involved in pathogenesis by facilitating bacterial erosion of cardiac surfaces , when the bacteria enter the blood as a consequence of trauma to oral tissue , (Smith ,*et al.,(*1994) and Labibe, *et al*.,(1997).The oral *Streptococcus* can produce a variety of proteolytic enzyme which include collagen-degrading enzyme ; eleastase like enzyme and tripsin –like enzyme.These enzymes are capable of degradation of structural periodontal tissue and protein involved in inflammatory and immune response (Cox ,*et al.*,1993 and Kesavalue , *et al.*,1996) .

Table (3-7) Protease production by *St. mutans*

Isolate No.	Protease production	Site isolation of
1	-	Dental plague
2	-	Dental caries
3	-	Dental plague
4	+	=
5	-	Dental caries
6	+	Dental plague
7	-	Dental caries
8	-	Dental plague
9	+	Dental caries
10	+	Dental plague
11	-	=
12	-	=
13	+	=
14	-	=
Total %	35.7 %	

3.4.1.3 Detection the ability of *St. mutans* for production of dextranase enzyme

Twenty four isolate of *St. mutans* were isolated from patients with (dental caries and periodontal diseases) were subjected for its ability to produce this enzyme after growth in culture medium containing 1 % dextran polymer. After (24 -48hr) of incubation at 37 C ,benedict reagent was added , only 8 isolates (33.3%) of *St. mutans* were found to produce dextranase enzyme as shown in table (3-8) .

This result was compatible with other studies that considerd *St. mutans* are predominant producers of dextranase enzyme (Colby and Russell , 1997) ;Igarashi ,*et al.*, (2002) ; Khalikova ,*et al.*, (2005) ;,who had found that this bacteria has ability to produce dextranase enzyme which decompose the dextran by dissolving α -1,3 and α -1,6 glycoside bonds that holding the glucose polymer in dextran molecule .

Dextran is the most important extra cellular bacterial polysaccharide. it can produced from sucrose in diet and influences dental plague formation. It acts as matrix of this plague ,that lead to development of dental caries and periodontal diseases .(Igarashi ,*et al.*, 2004).

Ability of *St. mutans* for production of dextranase was attributed to the ability of *St. mutans* to produce dextranase when the exogenous source of sugar (sucrose from diet) is reduced. Cariogenic *St. mutans*

will produce this enzyme to break down the dextran polymer in dental plague into glucose subunit and use this glucose as a sole source of carbon (nutrition requirement) ,so *St. mutans* still produce lactic acid even when the fermentable sugar was not taken , this acid causes dissolving of the enamel and dentin under low PH ,so *St. mutans* will continue producing dental caries even after thorough cleaning of teeth Todar ,2008) .

In general clinical application of this enzyme in treatment of dental disease as anti plague agent is due to its ability of removal dental plague by degradation dextran matrix of dental plague this lead to reduce accumulation of bacteria around the teeth , on the other word it will reduce dental caries and periodontal disease (Khalikova ,*et al.*, 2005) . Recently ,dentifrices (tooth pastes) have been developed which provide for enzymatic decomposition of dextran in dental plague (Simonson ,*et al.*,1994) .

Table(3-8) Dextranase produce by *St. mutans*.

Isolate No.	Dextranase production
1	-
2	+
3	-
4	-
5	-
6	+
7	-
8	-
9	+
10	+
11	-
12	-
13	-
14	+
15	-
16	-
17	+
18	-
19	+
20	-
12	-
22	-
23	+
24	-
Total %	33.3 %

3.4.1.3.1 Effect of sucrose on Dextranase enzyme production

Sucrose is one of disaccharide sugars ,that composed from two monosaccharide sugars , fructose and glucose sub unit. It is the most important cariogenic sugar presents in human diet.

In this study , the effect of sucrose at different concentrations (0.1 - 1.0 mg/ml) on dextranase production by *St. mutans* has been investigated. The result showed that the presence of sucrose in the media of dextranase productivity has no effect on enzyme synthesis. These results were attributed to that the sucrose sugar is a disaccharide and its hydrolysis will produce glucose that used by *St. mutans* for synthesis of extra cellular polysaccharide (dextran polymer) which promotes production of dextranase enzyme by *St. mutans* bacteria. These result was nearly compatible to the result obtained by Simonson ,*et al.,(*1994) , who used cariogenic *St. mutans* was inoculated on liquid media containing 10 % of sucrose to promote production of insoluble dextran in presence of sucrose .

3.4.1.3.2 Effect of Some Antibiotics On Dextranase Production

An-attempt was carried out to study the effect of some antibiotics(Ampicillin ,Cefotaxime , Amoxiclave and Tetracyclin) on production of dextranase by *St. mutans* had been investigated at concentration (0.1mg/ml) of antibiotics that added to M9 medium containing 1 %

dextran .The results revealed that the highest inhibition effect on dextranase production by antibiotics was recorded by Tetracycline (75 %) , while the lowest effect was recorded by the rest antibiotics (Amoxiclave and Ampicillin were have inhibition rate (38.5 %) and Cefotaxime (25 %) as shown in table(3-9)..

The mechanism of action of these antibiotics on dextranase enzyme production , in general is unknown . However , it may be due to that these antibiotics effect on gene expression of dextranase enzyme (dex gene) by *St. mutans* ,that may interpret the results above about the percentage of inhibition for dextranase production by these four antibiotics ,especially to high inhibition effect of Tetracycline. (Derwent , 1988)

On the other hand , the low effect of inhibition by rest antibiotics may be due to that these antibiotics have effect only on growth but not on dextranase production (Hanada, *et al.,* 2001).

Nearly the same results recorded by Igarashi ,*et al.,(*2004) ,who had noticed marked decrease in dextranase production following addition of inhibitor to the media designated for production of dextranase . also Derwent ,(1988) who found that the exepression of certain genes on *St. mutans* chromosome for production α -1,3 glucogenase was inhibited by Tetracycline mouth rinse .

In general there is very few studies about the effect of some antibiotics on dextranase production by *St. mutans* when grow in media contain dextran as inducible .

Also there are no previous studies about the effect of Tetracycline ,Cefotaxime ,Ampicillin and Amoxiclave . This study is considered as first study that investigated the effect of these antibiotics on dextranase enzyme production in the area of the study.

Table(3-9) The effect of some antibiotics on dextranase production

Isolate No.	Dextranase production				
	Without antibiotic	With antibiotic			
		AMP	AMC	CTX	TE
1	produced	–	–	_	+
2	=	–	–	_	–
3	=	+	–	+	+
4	=	–	+	_	+
5	=	+	+	+	+
6	=	+	–	_	+
7	=	–	+	_	–
8	=	–	–	_	+
Total %	100 %	38.5 %	38.5 %	25 %	75 %

* + effective

* * - non effective

3.5 Measurement of secretory immunoglobulin A (SIgA) concentration in saliva.

In this study , the concentration of sIgA separated from saliva by using poly ethylene glycol was investigated . Saliva were collected from patients with dental caries and some samples were collected from healthy subjects with no caries lesion.The results of present study , revealed that the mean concentration of salivary immunoglobulin A in patients were 13.8µg/ml ,while the mean concentration of control groups were 8.98 µg/ml (Figure 3-2). These results showed the concentration of sIg A in patients with dental caries were significantly higher than healthy persons ($P \leq 0.05$). These results attributed to prolong exposure to the cariogenic *St. mutans* Ag that lead to antigenic stimulation which causes increased production of sIgA from mucosal plasma cell.These results were compatible with Jean-San Chia ,*et al.,* (2001), had mentioned that Secretory immunoglobulin A (sIgA)-mediated protection against dental caries has been focused on interference with *St. mutans* adherence and inhibition of virulence factors involved in colonization.

St. mutans cell surface protein antigen I/II (AgI/II) and glucosyltransferases (GTFs) are ideal candidates for a dental caries vaccine because of their essential role in bacterial adherence and vulnerability to blocking by S-IgA in saliva. An ideal approach to a dental caries vaccine is to develop subunit vaccines that induce S-IgA against protective epitopes in AgI/II or GTFs. Protective epitopes are those molecular domains associated with adhesion and colonization

and which are accessible to antibody. A subunit vaccine also precludes the induction of antibodies to irrelevant or unwanted epitopes. Toward this goal, protective B-cell of AgI/II have been mapped in selected human populations analogous studies sIg A in saliva is limited (Kelly,*et al.,* 1995) . The recent study indicate that S- Ig A in saliva act as specific immunoglobulin against the those antigen which cause its production (Parslow,*et al.*,2001) .Presence the of *St. mutans* antigen in saliva in case of dental caries lead to induction for production of IL -8 and IL -4, these interleukins increase production of S-Ig A from oral mucosal plasma cell that act secretary immune system against infections (Al-Saadi ,(1998) ;Russel , (1999) .

Ruchel ,*et al* .,(2007**)** .investigated the salivary antibody reactivity with *St. mutans* antigens in children and they showed that the reactive IgA antibodyThus, natural salivary IgA antibody responses to GbpB at an early age may account, in part, for resistance to *St. mutans* infection , the although patterns of IgA specificities to *St. mutans* antigens high significantly who had been investigated in during screening for salivary IgA antibody to six putative immunodominant GbpB epitopes.

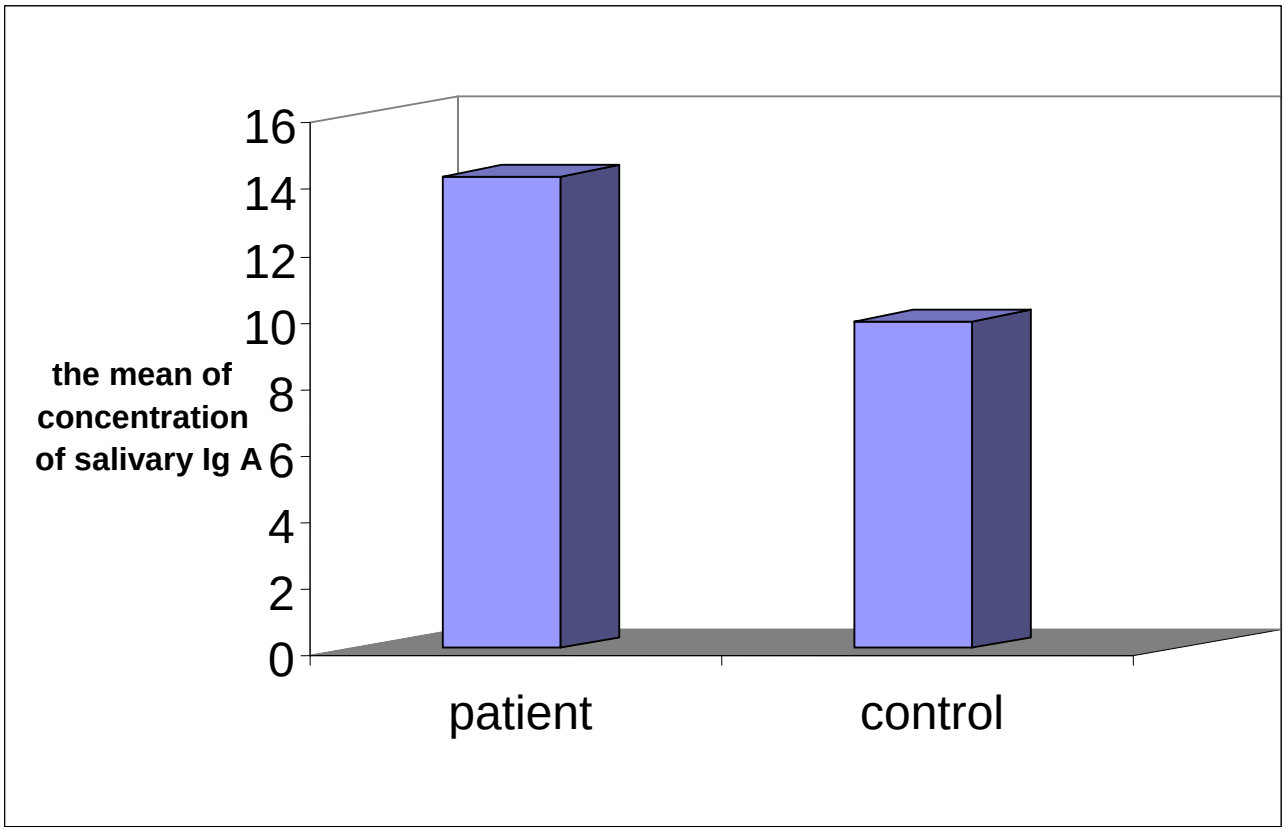

Figure (3-2) The mean concentration of salivary sIgA in patients with dental caries infection and control groups.