ORAL INFLAMMATORY CONDITIONS AND DIABETES MELLITUS

Fawad Javed

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OPPONENT:

Professor Palle Holmstrup, D.D.S, Dr. Odont., Department of Periodontology, School of Dentistry, University of Copenhagen, Copenhagen, Denmark.

EXAMINING COMMITTEE:

Professor Lennart Emtestam, M.D., Ph.D., Institute of Medicine, Department of Dermatology, Karolinska University Hospital at Huddinge, Stockholm, Sweden.

Docent Eva Toft, M.D., Ph.D., Institute of Medicine, Department of Endocrinology and Diabetology, Karolinska University Hospital at Huddinge, Stockholm, Sweden.

Docent Ola Norderyd, D.D.S, Ph.D., Department of Periodontology, Institute of Odontology in Jönköping, Jönköping, Sweden.

SUPERVISORS:

Main Supervisor: Docent Per-Erik Engström, D.D.S., Ph.D., Institute of Odontology, Department of Periodontology, Karolinska Institutet, Huddinge; and Department of Laboratory Medicine, Division of Clinical Immunology, Karolinska University Hospital at Huddinge, Stockholm, Sweden.

Co-Supervisor: Professor Björn Klinge, D.D.S., Odont. Dr., Institute of Odontology, Department of Periodontology, Karolinska Institutet, Huddinge, Sweden.

Authors’ addresses:

**Dr. Fawad Javed**

*Address I:*
Karolinska Institutet,
Institute of Odontology, Dept. of Periodontology
P.O.Box 4064, SE 141 04. Huddinge.
SWEDEN
Email: fawad.javed@ki.se
Ph: (+46) 70 430 4909

*Address II:*
Altamash Institute of Dental Medicine
2-R, Sunset Boulevard,
D.H.A. Phase IV. Karachi.
PAKISTAN
Email: fawadjaved19@yahoo.com
Ph: (+92) 300 211 1938

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Inflammation

Diabetes Mellitus
“Success is not measured by what you accomplish but by the opposition you have encountered, and the courage with which you have maintained the struggle against overwhelming odds.”

Orison Swett Marden

(1850-1924)
This thesis is dedicated to:

My parents, my grandmother and to the 
loving memory of my grandfather
ABSTRACT

The association between periodontal inflammation and diabetes mellitus has been reported. However, the influence of hyperglycemia and gender in oral inflammation is still unclear. In this thesis, periodontal conditions (plaque index [PI], bleeding on probing [BOP], probing pocket depth [PD] [4mm < 6mm and ≥ 6mm]), marginal bone levels on panoramic radiographs, oral yeast colonization, salivary protein concentrations and perceived oral symptoms were investigated in individuals with and without diabetes mellitus. Other variables that have been assessed include socioeconomic status (SES), education, gender, smoking, and gutka-chewing. Individuals aged between 45-64 years (Studies I, II and IV) and 10-19 years (Study II) were included in the research project.

Study I aimed to compare periodontal condition and socioeconomic status between subjects with and without type 2 diabetes (T2D).

Study II investigated the self-perceived oral health and the levels of salivary proteins in children with and without type 1 diabetes (T1D).

Study III aimed to investigate the periodontal conditions and oral symptoms in gutka-chewers with and without T2D.

Study IV evaluated the association between periodontal conditions, oral Candida albicans (C. albicans) colonization and salivary proteins in subjects with T2D.

In study I, PI, BOP, PD (4mm < 6mm) and marginal bone loss were increased in subjects with poorly-controlled T2D compared with well-controlled T2D. Individuals with poorly controlled T2D had a lower SES compared to patients with well-controlled T2D.

In study II, self-perceived gingival bleeding (GB), bad breath and dry mouth were higher in children with T1D compared with controls. Children with poorly-controlled T1D more often perceived GB and bad breath compared to those with well-controlled T1D. Salivary IgG/mg protein and total protein concentrations were higher in children with T1D compared with controls. Salivary IgG/mg protein levels were also elevated in children with poorly-controlled T1D compared to those with well-controlled T1D.

In study III, periodontal conditions (PI, BOP, PD [4mm < 6mm]) and oral symptoms were severe in gutka-chewers compared to non-chewers in non-diabetic subjects. In subjects with T2D, there was no difference in these variables between gutka-chewers and non-chewers.

In study IV, periodontal and salivary markers of inflammation (BOP and IgG/mg protein) were increased in type 2 diabetic females with C. albicans colonization compared with males.

In conclusion, this thesis shows that radiological and clinical parameters of periodontal destruction are increased in subjects with poorly-controlled T2D, and a poor SES aggravates the periodontal conditions in these subjects. Self-perceived GB and salivary IgG/mg protein levels are higher in children with T1D compared with controls. In subjects without T2D, gutka-chewing is associated with severe periodontal conditions and oral symptoms; however, in subjects with T2D, the severity of these variables is related to glycemic levels rather than gutka consumption. Periodontal and salivary markers of inflammation, BOP and IgG/mg protein, are increased in type 2 diabetic females with C. albicans colonization compared with males.
**Key words**: bleeding gums; marginal bone loss; periodontal conditions; *gutka*; IgA/mg protein; IgG/mg protein; saliva; smoking; socioeconomic status; type 1 diabetes; type 2 diabetes
LIST OF PUBLICATIONS

Comparison of periodontal and socioeconomic status between subjects 
with type 2 diabetes mellitus and non-diabetic controls. 

II. Javed F, Sundin U, Altamash M, Klinge B, Engström PE. 
Self-perceived oral health and salivary proteins in children with type 1 
diabetes. 
*Journal of Oral Rehabilitation*. 2008; Published online: October 13.

III. Javed F, Altamash M, Klinge B, Engström PE. 
Periodontal conditions and oral symptoms in *gutka-chewers* with and 
without type 2 diabetes. 

Periodontal conditions, oral *Candida albicans* and salivary proteins in 
type 2 diabetic subjects. 
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<th>Full Form</th>
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<tr>
<td>A. actinomycetemcomitans</td>
<td>Aggregatibacter actinomycetemcomitans</td>
</tr>
<tr>
<td>AGEs</td>
<td>Advanced glycation end products</td>
</tr>
<tr>
<td>AN</td>
<td>Areca nut</td>
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<tr>
<td>ANOVA</td>
<td>One-way analysis of variance</td>
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<tr>
<td>B. forsythus</td>
<td>Bacteroides forsythus</td>
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<tr>
<td>BOP</td>
<td>Bleeding on probing</td>
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<td>C. albicans</td>
<td>Candida albicans</td>
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<tr>
<td>CEJ</td>
<td>Cemento-enamel junction</td>
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<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>FDI</td>
<td>Fédération Dentaire Internationale</td>
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<tr>
<td>GB</td>
<td>Gingival bleeding</td>
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<td>GCF</td>
<td>Gingival crevicular fluid</td>
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<td>GDM</td>
<td>Gestational diabetes mellitus</td>
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<tr>
<td>g/L</td>
<td>Grams per litre</td>
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<td>HbA1c</td>
<td>Glycated haemoglobin A1c</td>
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<td>Ig</td>
<td>Immunoglobulin</td>
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<tr>
<td>IgA/mg protein</td>
<td>IgA per milligram of salivary total protein concentration</td>
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<tr>
<td>IgG/mg protein</td>
<td>IgG per milligram of salivary total protein concentration</td>
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<td>IL</td>
<td>Interleukins</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
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<tr>
<td>mm</td>
<td>Millimetre</td>
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<tr>
<td>mg/dl</td>
<td>Milligram per decilitre</td>
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<tr>
<td>mg/L</td>
<td>Milligrams per litre</td>
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<tr>
<td>mmol/L</td>
<td>Millimole per litre</td>
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<tr>
<td>MBL</td>
<td>Marginal bone loss</td>
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<tr>
<td>MT</td>
<td>Missing teeth</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PD</td>
<td>Probing pocket depth</td>
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<tr>
<td>PI</td>
<td>Plaque index</td>
</tr>
<tr>
<td>PMNL</td>
<td>Polymorphonuclear leukocytes</td>
</tr>
<tr>
<td>P. gingivalis</td>
<td>Porphyromonas gingivalis</td>
</tr>
<tr>
<td>RBGL</td>
<td>Random blood glucose level</td>
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<tr>
<td>SES</td>
<td>Socioeconomic status</td>
</tr>
<tr>
<td>SFR</td>
<td>Salivary flow rate</td>
</tr>
<tr>
<td>T1D</td>
<td>Type 1 diabetes</td>
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<td>T2D</td>
<td>Type 2 diabetes</td>
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<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor-alpha</td>
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<td>UWS</td>
<td>Unstimulated whole saliva</td>
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<tr>
<td>Glossary Term</td>
<td>Definition</td>
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<tr>
<td>Acute inflammation</td>
<td>Type of inflammation showing rapid onset, short duration, and dramatic signs and symptoms.</td>
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<tr>
<td>Antibody</td>
<td>Antibodies are a type of protein, produced by the immune system in response to harmful foreign substances such as bacteria, viruses, chemicals and/or toxins.</td>
</tr>
<tr>
<td>Antigen</td>
<td>An antigen is any substance that causes the immune system to produce antibodies against it. It may be a foreign substance from the environment such as bacteria, viruses, chemicals and/or toxins.</td>
</tr>
<tr>
<td>Cementoenamel junction</td>
<td>The area at which the enamel and cementum are united at the cervical region of the tooth.</td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>A common form of inflammation characterized by an absence of cardinal signs and symptoms, with the presence of lymphocytes, macrophages and fibroblasts.</td>
</tr>
<tr>
<td>Cytokine</td>
<td>Non-antibody proteins released by one cell population (e.g., T lymphocytes) on contact with specific antigen, which act as intercellular mediators, as in the generation of an immune response. Examples include lymphokines and monokines.</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>A chronic metabolic disorder caused by absolute or relative insulin deficiency.</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>A disorder involving inflammation of the gums (gingiva).</td>
</tr>
<tr>
<td>Immunoglobulin</td>
<td>Any of the structurally related glycoproteins that function as antibodies, divided into five classes (IgM, IgG, IgA, IgD, and IgE) on the basis of structure and biological activity.</td>
</tr>
<tr>
<td>Incidence</td>
<td>The rate at which a certain event occurs, e.g., the number of new cases of a specific disease occurring during a certain period in a population at risk.</td>
</tr>
<tr>
<td>Inflammation</td>
<td>A localized protective response elicited by injury or destruction of tissues, which serves</td>
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</tbody>
</table>
to destroy, dilute, or wall off (sequester) both the injurious agent and the injured tissue. It is characterized in the acute form by the classical signs of pain (dolor), heat (calor), redness (rubor), swelling (tumor), and loss of function (functio laesa).

**Insulinopenia**
A reduction in insulin.

**Periodontitis**
A dental disorder that results from progression of gingivitis, involving inflammation and infection of the ligaments and bones that support the teeth.

**Polydipsia**
A chronic excessive thirst and intake of fluids.

**Polyurea**
Passage of a large volume of urine in a given period.

**Prevalence**
The number of people in a population who have a disease or other condition at a given time.

**Reliability**
The tendency of a system to be resistant to failure.

**Risk factor**
A clearly defined occurrence or characteristic that has been associated with the increased rate of a subsequently occurring disease.

**Sensitivity**
The conditional probability that a person having a disease will be correctly identified by a clinical test, i.e., the number of true positive results divided by the total number with the disease (which is the sum of the numbers of true positive plus false negative results).

**Socioeconomic status**
A measure of an individual or family’s relative economic and social ranking.

**Validity**
The extent to which a measurement, test, or study measures what it purports to measure.

**Xerostomia**
The condition of not having enough saliva to keep the mouth wet due to inadequate function of the salivary glands.
Figure 1. Thesis at a glance
INTRODUCTION

This thesis focuses on the association between oral inflammatory conditions and diabetes mellitus (DM). The number of diabetic individuals is increasing worldwide because of population growth, aging, increasing prevalence of obesity and urbanization. It has been reported that the worldwide prevalence of DM will rise from 171 million (in 2000) to 366 million by 2030 (Wild et al, 2004). The complications of DM include cardiovascular disorders, retinopathy and peripheral nerve abnormalities (Eppens et al, 2006). The most common oral complication of DM is periodontal disease, which has also been labeled as the “sixth” complication of DM (Løe, 1993). The reason for an increased incidence of periodontal inflammation in diabetic subjects is unclear; however, alterations in host-pathogen response in diabetic patients may intensify inflammation in the periodontal tissues.

ORAL BIOFILM (DENTAL PLAQUE)

Regular oral hygiene maintenance supports bacterial control and helps to maintain a comparatively constant composition of healthy bacteria (Haffajee et al, 2006). Clinically, this bacterial control correlates with an absence of visible inflammation (hemorrhagic gingiva) and pocket depths of less than 3 mm (Johnson and Serio, 2001). Biofilms are bacterial communities composed of several different organisms existing in a collective state. The oral biofilm or dental plaque is characterized by large numbers of anaerobes, spirochetes and motile bacterial species (Haffajee et al, 2006). An extracellular environment is created with an identifiable structured organization of bacteria, in which nutrients and defenses become shared. Biofilms are characterized by impermeability and therefore resistant to host molecular and cellular defenses, as well as to chemotherapeutics (Socransky and Haffajee, 2002). In this environment, known periodontal pathogens are able to flourish and reproduce, and the disease progresses. If bacterial colonization in the biofilm exceeds a threshold level, the immunological defense will react by initiating a series of events in the underlying connective tissues. Clinically, this correlates with increased clinical signs of inflammation (gingival hemorrhage) and increased pocket depths (Socransky and Haffajee, 2002). At a cellular level, this is characterized by an increase in certain inflammatory parameters, cellular breakdown products, and sulcular fluid flow.
INFLAMMATION

Inflammation (Latin, *inflammatio*, to set on fire) is the complex biological response of vascular tissues to harmful stimuli, such as pathogens or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as to initiate the healing process of the tissue. Inflammation may be defined as "a localized protective response, elicited by injury or tissue destruction, which serves to obliterate both the injurious agent and the injured tissue.

**Table 1.** Comparison between acute and chronic inflammation

<table>
<thead>
<tr>
<th></th>
<th>Acute inflammation</th>
<th>Chronic inflammation</th>
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<tbody>
<tr>
<td><strong>Etiology</strong></td>
<td>Pathogens, tissue injury</td>
<td>Persistence of acute inflammation</td>
</tr>
<tr>
<td><strong>Major cells involved</strong></td>
<td>Neutrophils, monocytes, macrophages</td>
<td>Monocytes, macrophages, lymphocytes, plasma cells, fibroblasts</td>
</tr>
<tr>
<td><strong>Onset</strong></td>
<td>Immediate</td>
<td>Delayed</td>
</tr>
<tr>
<td><strong>Duration</strong></td>
<td>Few days</td>
<td>Up to many months or years</td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td>Healing, abscess formation, chronic inflammation</td>
<td>Tissue destruction, fibrosis</td>
</tr>
</tbody>
</table>

There are two fundamental types of inflammation: *acute* and *chronic* inflammation. *Acute inflammation* is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as *chronic inflammation*, leads to a progressive shift in the type of cells which are present at the
site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process (Table 1).

Chemical mediators play a significant role during an inflammatory process. These inflammatory mediators come from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes/macrophages, B and T cell lymphocytes (Roger, 2004). They are triggered by bacterial products or host proteins. Chemical mediators bind to specific receptors on target cells and can increase vascular permeability and neutrophil chemotaxis, stimulate smooth muscle contraction, have direct enzymatic activity, induce pain or mediate oxidative damage. Examples of chemical mediators include prostaglandins and cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukins (IL).
PERIODONTAL INFLAMMATION

The tooth and its surrounding tissues communicate with the external environment by perforating through the oral epithelium. A healthy periodontium is characterized by dense connective tissues with infrequent number of leukocytes and a few layers of junctional epithelia in the gingival sulcus. The junctional epithelium acts as a seal thereby protecting the underlying connective tissues. The sulcus is colonized by mainly aerobic bacteria and is bathed in a continuous flow of plasma that passes through the junctional epithelium. The sulcular fluid also contains neutrophils, which eradicate bacteria through phagocytosis and degranulation.

Periodontal inflammation is a common manifestation worldwide affecting 10 to 15\% of most adult populations (Papapanou, 1999). Plasma cells and lymphocytes are principal inflammatory cells in chronic periodontal inflammation. It has been estimated that in subjects with moderate to severe periodontal inflammation, the total surface area of the inflamed periodontal pockets may range from 8 to 20 cm\(^2\), depending on the number of dentition affected (Hujoel, 2001). Consequently, the large surface area of the periodontal tissues involved depends on the severity of the host-induced inflammatory response.

Pathophisiology

There is considerable evidence that microbes, including Porphyromonas gingivalis (P. gingivalis), Bacteroides forsythus (B. forsythus) and Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans) have a direct relationship with periodontal inflammation (Liljengerg et al, 1994, Consensus Report, 1996; Ximenez-Fyvie et al, 2006; Bidault et al, 2007, Van Dyke, 2008). Such microbes are resistant to the normal host defense system; they spread laterally and apically along the root surface, causing tissue destruction and pocket deepening (Page et al, 1997; Page, 1998). These microorganisms synthesize toxins that induce a host-mediated tissue destructive immune response (Offenbacher, 1996). These toxins include lipopolysaccharides and proteases that can trigger tissue destruction and degrade specific antibodies in the host defense mechanism. This may be an explanation for the recurrence of inflammation in
subjects with high titers of salivary and serum antibodies specific for the antigens of their infecting microbes.

If the microbial challenge persists, the microbes and their products penetrate the connective tissues, which results in the formation of a periodontal pocket. The pocket epithelia provide access for bacterial products to bathe the connective tissues, as a result, cause epithelial cells express pro-inflammatory cytokines. This causes edema and results in the formation of an inflammatory cell infiltrate. If repression of the microbes does not occur, inflammation worsens and may lead to tissue destruction and alveolar bone loss. Macrophages produce cytokines including IL-1 beta (β), IL-6, IL-8 and TNF-α, which play a significant role in periodontal bone resorption (Page et al, 1997).

Markers in periodontal inflammation

Gingival inflammation, if not controlled or treated may progress to deeper connective tissues resulting in the loss of periodontal ligament and supporting alveolar bone. The gingival crevices are the main source of salivary immunoglobulin (Ig) G, and since the gingival fluid increases during inflammation, it is most likely that subjects with periodontal inflammation will show high titers of IgG in the saliva. Increased levels of salivary IgG and IgA have been reported in subjects with periodontal inflammation (Anil et al, 1995). Besides salivary antibodies, total salivary protein concentration may also be increased as a result of periodontal inflammation. A significant positive correlation has been found between periodontal inflammation and the concentrations of salivary proteins, including albumin, lactoferrin, lysozyme and myeloperoxidase (Ben-Aryeh et al, 1993; Dodds et al, 2000). Other markers of periodontal inflammation and bone destruction include TNF-α, IL’s and matrix metalloproteinases (Heasman, 1993; Hayashi et al, 1994; Yamamoto et al, 1997; Bretz, 2005, Van Dyke and Kornman, 2008).
Risk factors

• **Age**

  Aging has been associated with periodontal inflammation (Norderyd & Hugoson, 1998; Ebersole et al, 2008). The longer exposure to microbes during a life span increases the likelihood of the development of inflammatory conditions in the body (Genco, 1996). Almost three decades ago, it was determined that periodontal inflammation was most prevalent by middle age. It remains unclear whether becoming older is related to increasing periodontal inflammation or if the worsening periodontal status is related to the consequences of aging. However, it has been documented that the rate of periodontal destruction may increase after the age of 70 years (Machtei et al, 1994). Advancing age has also been associated with the accumulation of advanced glycation end products (AGEs) in the plasma and tissues, which are associated with periodontal destruction (Wautier and Guillausseau, 2001).

• **Smoking**

  There is a positive association between smoking and periodontal inflammation (Geismar et al, 2006). Tooth loss in adults aged 19 to 40 years is associated with smoking (Holm, 1994). Nicotine in tobacco smoke constricts the gingival blood vessels, which accounts for a reduced bleeding on probing (BOP) (Black et al, 2001). Simultaneously, salivary albumin and IgG levels have also been shown to be reduced in smokers compared to non-smokers (Nagler, 2007). This reflects that smoking tends to mask the signs of the ongoing inflammation. Periodontal treatment is probably less successful in smokers and the disease is more likely to recur than in comparison to non-smokers (Preber and Bergström, 1990). Smoking also enhances the release of IL-1β, which impairs the function of osteoblasts (Payne et al, 1996).
• **Gender**

Gender is significantly associated with oral health status. It has been reported that clinical signs of periodontal inflammation are more evident in males compared with females (Grossi et al, 1995). A recent study reported that female gender was notably associated with oral hygiene maintenance and visits to the dentists (Bertea et al, 2007). Variations in hormonal levels among females may alter the periodontal health status (Guthmiller et al, 2001). Menopause has been related with periodontal bone loss and the reduction in estrogen levels raise the possibility of bone resorption around the teeth (Cenci et al, 2003). The mean age of Pakistani females at menopause is 47.1 years (Baig and Karim, 2006), compared to Sweden, where menopause begins at the age of approximately 50 years (Nedstrand et al, 1995).

• **Diabetes Mellitus**

Subjects with poorly-controlled type 1 diabetes (T1D) and type 2 diabetes (T2D) are more prone to periodontal inflammation compared with non-diabetic individuals (Thorstensson and Hugoson, 1993; Seppälä and Ainamo, 1994; Geismar et al, 2006; Demmer et al, 2008). DM results in changes in the function of immune cells, including neutrophils, macrophages and monocytes, which allow the microbes to persist in periodontal pockets thereby increasing the possibility for periodontal destruction (Mealey, 2006). The gingival blood vessels of diabetic individuals have thickened basement membranes that lead to a reduction in transport across the vessel walls. There is also a reduction in collagen production by gingival and periodontal fibroblasts in subjects with DM (Seppälä et al, 1997). The underlying pathophysiology that increases the risk of periodontal inflammation in subjects with DM is poorly understood; however it has been associated with the accumulation of glucose-mediated AGEs.

AGEs, such as $N$-carboxymethyl-lysine, pentosidine and methylglyoxal derivatives, constitute a varied group of molecules formed by the non-enzymatic reaction of reducing sugars and carbohydrates (Peppa and Vlassara, 2005). AGEs accumulate during the process of normal aging in the plasma and tissues, but to an accelerated degree in patients with DM (Wautier and Guillausseau, 2001). AGEs may
lead to tissue damage in two ways: (1) they may distress the function of lipids and proteins; and/or (2) may interact with specific cell-surface receptors for AGEs and cause an alteration in intracellular events. This may lead to inflammation. (Peppa and Vlassara, 2005).

**Figure 2.** Cycle of periodontal damage in diabetes. Modified from: Cronin et al (2008).
• *Obesity*

Obese subjects are predisposed to a variety of complications that affect overall well-being including periodontal health. In Copenhagen, Denmark, obesity, an important risk factor for T2D is significantly higher among Pakistani, Turkish and Arab individuals (Pearson et al, 2007). Although, the biological mechanism for the association between periodontal inflammation and obesity remains to be established, it is known that adipose tissues secrete a number of cytokines and hormones that are involved in the inflammatory process (Pischon et al, 2007). However, socioeconomic factors may also be associated with the above mentioned relationship as obesity is more prevalent among subjects being of a poor socioeconomic status (SES).
Figure 3. Geographic location of the countries grouped according to the gross domestic product per capita classification used by the World Bank. (Corbet et al, 2002). (This figure was reprinted with permission from Wiley-Blackwell).
Discrepancy in health status between social classes has persisted over time. The term “SES” is used to describe factors about an individuals' lifestyle, including occupation, income and education level. Subjects of different SES may have very different access to medical care, healthy food, and physical activity opportunities. Individuals with a deprived SES have poorer health and are less likely to have adequate access to healthcare and/or prevention care services compared to subjects with privileged living standards (Krieger et al, 1997). These factors may affect health and should be accounted for when studying risk of different diseases and conditions.

The relationship between SES and health has been extensively documented. Studies have shown that there is an association between deprived living conditions and T2D (Evans et al, 2000; Javed et al, 2007). In the United States, females with T2D are twice as likely to have a lower SES compared with non-diabetic women (Centers for Disease Control and Prevention, 2002). It has recently been reported that under-privileged living standards are associated with DM, poor self-rated health and depression (Demakakos et al, 2008). In Copenhagen, Denmark, a deprived SES is significantly higher among Pakistani, Turkish and Arab individuals (Pearson et al, 2007). In addition, a study from Pakistan reflected that reports of poor health are more common among subjects with a poor SES (Ahmad et al, 2005).

An underprivileged SES has also been associated with poor oral health. A recent study has shown that periodontal inflammatory conditions are higher in subjects with a lower SES (López and Baelum, 2007). In another study, high-school children whose fathers’ had lower salaries were more susceptible to have periodontal inflammation compared with fathers of students with higher income statuses (López et al, 2006).

Literacy level in individuals depends on several factors, including location of individuals in a community, social status, age, gender and ethnicity. It has been reported that illiteracy and hyperglycemia are more prevalent among Pakistani females with T2D compared to males (Hawthorne and Tomlinson, 1999). Besides DM, poor education is also an inevitable risk factor for stroke (Tseng et al, 2005). Education level is a representative of SES, which is associated to quality of life in diabetic individuals (Ghanbari et al, 2005). A recent Danish study showed that the risk of
having a positive parental history of T2D was 13 times higher in subjects with a Pakistani ethnicity compared to ethnic Danish individuals (Pearson et al, 2007).
ASSOCIATION BETWEEN PERIODONTAL INFLAMMATION AND DIABETES MELLITUS

Clinical and epidemiological studies have demonstrated that subjects with T1D, as well as T2D, have a higher prevalence and severity of periodontal inflammation compared with non-diabetic individuals (Emrich et al, 1991; Safkan-Seppälä and Ainamo, 1992; Tenenbaum et al, 2007). Simultaneously, the severity of periodontal inflammation is increased in subjects with poorly-controlled DM compared to individuals with well-controlled DM. However, individuals with a good metabolic control of their diabetes may also display inflammation in periodontal tissues (Seppälä and Ainamo, 1994). This may propose that some diabetes induced mechanisms tend to weaken the host resistance. It may be suggested that there are basically two ways in which diabetes may influence periodontal inflammation:

- Production of AGEs due to hyperglycemia.
- Alteration in oral microbiota.

In diabetic individuals, especially those with hyperglycemia, the severity of periodontal conditions is further amplified by the accumulation of AGEs (Peppa and Vlassara, 2005). It has been reported that in diabetic subjects the amount of marginal bone loss (MBL) is independent of plaque conditions and is majorly influenced by hyperglycemia (Yalda et al, 1994). Furthermore, other studies have demonstrated that hyperglycemia is mainly responsible for escalating the severity of periodontal conditions in subjects with T2D (Yalda et al, 1994). On the other hand, T1D has been associated with certain genetic factors that cause an increased production of inflammatory mediators; in fact hyperglycemia may play a minor role in aggravating the periodontal status (Yalda et al, 1994). Of course, the contribution of microorganisms in this context can not be over-looked, however it may be suggested that AGEs take the main credit for worsening the periodontal status in T2D subjects with hyperglycemia (Yalda et al, 1994).

It is well established that host-microbe interactions play a significant role in the initiation and progression of periodontal conditions (Van Dyke, 2008). Periodontal tissue destruction appears to result from a complex interplay between the...
host response and specific plaque microorganisms, such as \textit{P. gingivalis}, \textit{B. forsythus} and \textit{Treponema denticola} (van Winkelhoff et al, 2002). These bacteria possess an array of virulence factors, such as proteases, which are associated with periodontal inflammation. It has been reported that \textit{B. forsythus} and \textit{P. gingivalis} are the strongest bacterial markers for periodontal bone loss (van Winkelhoff et al, 2002). In subjects with DM, the potential pathogenic mechanisms for alveolar bone loss are accelerated due to factors such as impaired recruitment and function of neutrophils in response to infection with pathogenic bacteria, decreased collagen formation and exaggerated collagenolytic activity (Manouchehr-Pour et al, 1981; McMullen et al, 1981; Ramamurthy and Golub, 1983; Sasaki et al, 1992). However, it has also been documented that there is no association between periodontal pathogens and glycemic status in subjects with DM (Sbordone et al, 1995).

\textbf{ASSOCIATION BETWEEN PERIODONTAL INFLAMMATION AND GLYCEMIC LEVELS}

In addition to the large amount of evidence that show DM as a risk factor for periodontal inflammatory conditions, there is a growing number of facts, which demonstrate that periodontal inflammation adversely affects glycemic levels. The high vasculatiry of an inflamed periodontal tissue may make it a source for inflammatory mediators (Offenbacher et al, 1996). Mediators such as TNF-\(\alpha\), IL-6 and IL-1 have been shown to effect glucose and lipid metabolism by antagonizing insulin action (Taylor and Borgnakke, 2008). In subjects with severe periodontal inflammation, DM complications, such as cardiovascular disorders, are more intense compared to individuals with minor periodontal inflammation (Thorstensson et al, 1996). However, a meta-analysis of studies to quantify the effects of periodontal treatment on glycemic levels among subjects with T2D showed a non-significant decrease in glycemic levels following treatment of periodontal inflammation (Janket et al, 2005).
DIABETES MELLITUS

Definition, symptoms and types

The term diabetes is derived from the Greek word διαβαίνειν that means "passing through (urine)" and the word mellitus is derived from Latin, which means "honey". Diabetes mellitus means, literally, honey-sweet urine.

DM is a group of metabolic disorders characterized by hyperglycemia due to defects in insulin secretion, insulin action, or both (American Diabetes Association, 2004). Symptoms of DM include polydipsia, polyuria, impaired vision and weight loss (American Diabetes Association, 2003). Chronic hyperglycemia may also be associated with an impaired growth rate and increased susceptibility to infections (American Diabetes Association, 2003). If the immune system malfunctions, this inflammatory process can damage healthy tissue. Several markers of inflammation are increased in the serum of diabetic subjects. Cytokines such as TNF-α and IL’s, are secreted by islet cells (Katsuki et al, 1998; Ehses et al, 2008). Increased levels of inflammatory markers, including leukocyte count have also been associated with the future development of T2D (Vozarova et al, 2002). Hyperglycemia in diabetic subjects may also be associated with chronic vascular inflammation thereby inviting microvascular complications (Feng et al, 2005).

The main types of DM include T1D, T2D and gestational diabetes mellitus (GDM).

- Type 1 Diabetes

“Type 1” indicates the process of beta (β)-cell destruction that may lead to DM. It accounts for 5-10% of the subjects with DM and the rate of β-cell destruction varies from individual to individual (Zimmet et al, 1994). The rapidly progressing form of T1D is common amongst children but may also be seen in adults (Humphrey et al, 1998). Whereas, the slowly progressive form of T1D is reported to occur mainly only
in adults, where it is also known as “Latent autoimmune diabetes in adults”. The markers of immune destruction of β-cells include islets cell auto-antibodies and auto-antibodies against insulin. Autoimmune destruction of β-cells may be related to genetic as well as environmental factors, which still require clarification (American Diabetes Association, 2005). Certain environmental factors, such as viral infection, nutritional imbalances, low birth weight and parental age may also be associated with T1D (Akerblom et al, 2002). Although subjects with T1D are less often obese, the association of obesity with DM can not be over-looked. Besides systemic signs, including polyuria, weight loss, fatigue and excessive thirst, oral symptoms incorporate xerostomia, periodontal inflammation and candidial infections (Belazi et al, 1998).

- **Type 2 Diabetes**

  T2D accounts for approximately 90-95% of all diabetic individuals (American Diabetes Association, 2005). It is usually latent in its early stages and can remain undiagnosed for several years (American Diabetes Association, 2004). Subjects with T2D have a relative, rather than an absolute deficiency of insulin. The specific etiology of T2D is not known, however several different causes have been associated with T2D. The majority of patients with T2D are obese and obesity itself causes insulin resistance to some extent (Mokdad et al, 2001). A weakly defined genetic predisposition of T2D has also been suggested (Florez, 2008). The risk of developing T2D is directly associated with age, obesity and lack of physical exercise (American Diabetes Association, 2005).

- **Gestational Diabetes Mellitus**

  Any degree of glucose intolerance that is recognized during pregnancy is known as GDM (Kim et al, 2002). Females with GDM are susceptible to develop diabetes, frequently T2D, after pregnancy and the children of females with GDM are also prone to develop glucose intolerance, obesity and DM in childhood as well as adolescence (American Diabetes Association, 2003).
**Pre-diabetes**

Pre-diabetes is an intermediate phase between normal glycemic levels and the clinical entity of T2D. The presence of impaired fasting glucose and/or impaired glucose indicates the presence of pre-diabetes (Valensi et al, 2005). It has been defined as an impaired glucose tolerance (two hour glucose concentration 7.8-11 mmol/L after a glucose load) or an impaired fasting glucose concentration of 6.1-6.9 mmol/L (Bhopal and Fischbacher, 2002; Aroda and Ratner, 2008). The risks for progression of pre-diabetes to T2D, include degree of insulin resistance, age, family history, obesity, and/or history of GDM (Aroda and Ratner, 2008). The symptoms for pre-diabetes are often latent; however, it is essential to look for the classic signals of T2D such as frequent urination, impaired vision polydipsia, polyurea and fatigue.

A high prevalence of pre-diabetes (30.5%) has been reported in South Asian populations originating from the Indian subcontinent (Bhopal and Fischbacher, 2002). The prevalence of pre-diabetes in Pakistan and India has been reported to be 9.3% and 14.6% (Shera et al, 1995; Zargar et al, 2006; Mohan et al, 2008). The prevalence of pre-diabetes in Sri Lanka is 11.3% (Katulanda et al, 2008).

Periodontal inflammation is not just a hazard for subjects with DM but also for individuals with pre-diabetes (Pontes Andersen et al, 2007; Pontes Andersen et al, 2007). Periodontal inflammatory conditions may cause individuals to develop characteristics of pre-diabetes. Periodontal inflammation may agitate glucose regulation in non-diabetic subjects, with characteristics of pre-diabetes, which may later progress to T2D (British Dental Journal/News, 2007). It has also been hypothesized that in subjects with impaired glucose tolerance, the duration of hyperglycemia may be too short to initiate tissue changes (Sastrowijoto, 1990).
Epidemiology

It is calculated that presently there are approximately 150 million people with DM worldwide, and by the year 2025, this number may rise to 300 million (Zimmet et al, 2001). Over 9% (~21 million) of the American adult population has DM and it is estimated that approximately six million of these are undiagnosed diabetics (Mealey and Oates, 2006). In 1995, China, India and the United States were the "top three" countries estimated to have the highest numbers of people with DM, and in the list for 2000 and the predicted list for 2030, Bangladesh, Brazil, Indonesia, Japan, and Pakistan have also appeared (Wild et al, 2004).

The incidence of T1D in childhood varies between countries. Nordic countries, including Finland (36.5/100,000/year), Norway (22.4/100,000/year) and Sweden (21.1-31.9/100,000/year) have reported the highest rates of T1D in children aged less than 15 years (Tuomilehto et al, 1995; Dahlquist and Mustonen, 2000; Joner et al, 2004; Adeghate et al, 2006). In Canada and the United States, the incidences of T1D in children are approximately 25.7/100,000/year and 7.61-13.4/100,000/year respectively (Adeghate et al, 2006). Compared with Western countries, the incidence of T1D in Asia has been reported to be significantly low. In Pakistan, India and Iran, the estimated incidences of T1D are 1.0/100,000/year, 10.5/100,000/year and 4/100,000/year respectively (Ramachandran et al, 1996; Staines et al, 1997; Pishdad, 2005).

It is estimated that the present prevalence rate of T2D in Pakistan is 10% of the adult population (Shera et al, 1995; Shera et al, 1999; Nishtar, 2004). The prevalence of T2D in Sweden and Denmark have been reported to be about 3—4% and 4.2% respectively (Henriksson and Jönsson, 1998; Henriksson et al, 2000; Carstensen et al, 2008). According to another study, the prevalence of DM among Finnish males and females (aged between 45-64 years) is 10.2% and 7.4% respectively (Ylihärsilä et al, 1986). In two areas of Tehran (Iran), the prevalence of T2D has been reported as 8.7% and 14.5% respectively (Azizi et al, 2003).
Risk factors

Some of the risk factors that have been associated with DM are listed in Table 2.

Table 2. Common risk factors associated with T2D. (Modified from American Diabetes Association, 2004).

- Age ≥45 years
- Overweight (BMI ≥25 kg/m² *)
- Family history of diabetes (i.e., parents or siblings with diabetes)
- Habitual physical inactivity
- Race/ethnicity (e.g., African-Americans, Hispanic-Americans, Native Americans, Asian-Americans, and Pacific Islanders)
- Prediabetes
- History of GDM
- Hypertension (≥ 140/90 mmHg in adults)
- High cholesterol in blood

* May not be correct for all ethnic groups.

Diagnostic criteria

A single laboratory test is insufficient to diagnose DM, therefore any positive laboratory result should be re-confirmed on another day. A fasting blood glucose level of more than 126 mg/dl or a 2-hour post load glucose tolerance of more than (or equal to) to 200 mg/dl is used to diagnose DM (Kuzuya et al, 2002).

In diagnosed diabetic patients, the hemoglobin A₁c (HbA₁c) test is generally performed to screen the glycaemic control. Glucose forms a stable bond with hemoglobin, thus hemoglobin remains glycated to the erythrocytes for approximately 123 ± 23 days. The HbA₁c test provides a good estimation of the average blood glucose level over the previous 30 to 90 day period. HbA₁c levels correlate well with the development of diabetic complications and may be valuable in the diagnosis of DM (Mealey and Oates, 2006; Bennett et al, 2007). In pre-diagnosed diabetic patients,
random blood glucose level (RBGL) measurement is a practical method to check the blood glucose levels, and a recent study has reported that RBGL levels can be used to screen T2D (Lambert and Chapman, 2004; Pearson et al, 2007).
PANORAMIC RADIOGRAPHS

Panoramic radiography is a modified type of tomography or image layer radiography. There have been substantial discussions concerning the choice of radiographs for the assessment of periodontal disease. Although, periapical radiographs are commonly employed to gauge the MBL in periodontal disease, albeit, panoramic radiography can be used as an alternative (Molander et al, 1991). Panoramic radiographs offer an exclusive radiographic view, covering the entire dentition and surrounding anatomical structures including the facial bones, condyles, parts of the maxillary sinus and nasal complexes. The technique is less time consuming and is comfortable for the subject. The radiation dosage is also significantly lower in panoramic radiographs compared with periapical radiographs. There is an association between bone loss, as observed on panoramic radiographs and clinical periodontal assessment (Walsh et al, 1997). Therefore, panoramic radiographs can be substituted for full-mouth intra-oral radiographs (Persson et al, 2003; Li et al, 2007).

Measurement of marginal bone levels using computer-aided programs

Manual instruments, such as Vernier calipers, are commonly employed to gauge marginal bone levels. However, computer-aided image analysis programs may be beneficial compared with the use of hand-held instruments. Computer-assisted measurement of MBL offers a high level of accuracy and reliability (De Smet et al, 2002). Computer-aided radiographic analysis tools can be used to detect and visualize early transformations in periodontal inflammatory conditions and programs have been used to construct three-dimensional models of alveolar bone, thereby providing accurate data concerning volumetric changes in the alveolar bone (Park et al, 2007). Another benefit of digital imaging is the ability to adjust the radiographic image using tools, such as brightness and contrast control, colorization, inversion and zooming (de Morais et al, 2006). Such features of computer-based imaging may help to detect minor bony changes that may otherwise remain unnoticed.
SALIVA

Saliva is a complex oral fluid comprising of a mixture of secretions from both major as well as minor salivary glands. Three pairs of salivary glands mainly produce saliva; namely, parotid, sub-mandibular and sub-lingual glands, in addition to several minor salivary glands. The daily production of saliva in humans is approximately 500-600 ml/day (Dawes, 2004) of serous and mucinous saliva that contains immunoglobulins, cytokines, electrolytes, minerals and other glycoproteins (Dodds et al, 2005).

Saliva can be considered as gland-specific saliva and unstimulated whole saliva (UWS). The gland-specific saliva can be collected individually from the individual salivary glands: parotid, sub-mandibular and sub-lingual gland. UWS may be defined as a mixture of oral fluids and includes secretions from the major and minor salivary glands, in combination with other constituents of non-salivary origin, such as gingival crevicular fluid (GCF), secretions from the respiratory tract, serum, desquamated epithelial cells and food debris (Kaufman & Lamster, 2002).

Salivary flow rates more than 0.2 ml/min are considered to be normal (Flink et al, 2005). Age influences salivary secretion, probably due to the physiologic process of ageing. It has been reported that older age is associated with decreased unstimulated whole salivary flow rate (SFR) (Fenoll-Palomares et al, 2004) and that SFR is reduced in females compared to males (Fenoll-Palomares et al, 2004). A possible explanation for this may be that the size of the salivary glands is smaller in females compared to males (Inoue et al, 2006). The SFR is significantly higher in smokers compared to non-smokers (Nagler, 2007). An increased duration and frequency of areca nut (AN) chewing habit increases the SFR (Rooban et al, 2006). However, there is no difference in SFRs between tobacco-chewers compared to non-chewers (Khan et al, 2003).
Humoral immunity is mainly based on B-lymphocytes and involves circulating *antibodies* or *immunoglobulins*. The B-lymphocytes secrete immunoglobulins after antigen recognition. The immune systems of mucous membranes have been widely discussed in connection to humoral immunity. The presence of antibodies in external secretions, such as saliva, tears, sweat, colostrum and gastrointestinal and bronchial secretions, acts as a vital resistance factor against foreign or antigenic substances.

Immunoglobulins comprise of five structural classes as shown in Figure 4.

![Figure 4. Classification of human immunoglobulins.](image-url)
The most abundant antibody in serum is IgG, accounting for about 80% of the entire Ig content. Of all the immunoglobulins, only IgG can cross the placental barrier and thus is the most important antibody class in protecting newborn infants against infection (Duncan, 1995). Compared with other immunoglobulins, IgG rapidly diffuses through the extravascular body spaces where it reduces bacterial toxins and binds to microorganisms thereby enhancing their phagocytosis. IgG is monomeric, is a “Y” shaped molecule and has a serum half-life of approximately 21 days (Seidel et al, 2000).

The concentration of IgG in normal saliva is approximately 20 mg/L (Engström et al, 1996; Engström et al, 1999), which is about 0.1% to 0.2%, of its serum concentration (Granade et al, 2002). In oral inflammatory conditions, for instance gingivitis and periodontal disease, salivary IgG may leak from the serum into the oral environment via the GCF. Smoking and tobacco chewing may lead to a variation in IgG levels in oral fluids (Dodds et al, 2000; Granade et al, 2002). IgG is divided into four subclasses namely IgG1, IgG2, IgG3 and IgG4. The proportions of IgG1, IgG2, IgG3 and IgG4 in saliva have been accounted to be 11.2 mg/L, 8.5 mg/L, < 2 mg/L and 4.9 mg/L respectively; whereas in serum, these proportions are 8.5 g/L, 5.7 g/L, 1.4 g/L and 0.74 g/L correspondingly (Engström et al, 1996).

IgA appears selectively in sero-mucous secretions, such as saliva, sweat, tears, colostrum, nasal fluids and gastro-intestinal, urogenital and bronchial secretions (Pitiphat et al, 2002). In saliva, IgA plays a significant role in protecting oral mucosal and teeth surfaces from bacteria, viruses and other invasive antigens. The concentration of IgA is approximately six times higher in UWS, compared with stimulated parotid saliva (Engström, 2002). The concentration of IgA in UWS ranges between 100-300 mg/L (Patinen et al, 1995; Sari-Sarraf et al, 2008). The daily systemic IgA production is 1295-2100 mg/day, while secretory IgA is produced at a rate of about 2303-7058 mg/day. However, the oral secretion of IgA is approximated to be 100 mg/day. In
humans, there are two subclasses of IgA, namely IgA1 and IgA2, which occur in similar proportions (60% IgA1 and 40% IgA2) in saliva and other secretions (Marcotte and Lavoie, 1998). The half-life of IgA is 5.0 to 6.5 days (Austin et al, 1987).

**IgM, IgD and IgE**

IgM is the earliest class of immunoglobulins to be detected on B-cells and is the third most common serum Ig. IgM is very efficient in leading to the lysis of microorganisms. The normal concentrations of IgM in serum and whole saliva have been reported to be 107 mg/dl and 0.6 mg/dl respectively (Plenabi et al, 1983). IgM has a half-life of 5-10 days.

The salivary, as well as serum concentration of IgD are extremely low, approximately, 1.4 mg/dl and 0.01 mg/dl respectively (Plenabi et al, 1983). The exact function of IgD is unclear. IgD has a half-life of approximately 2.8 days (Rogentine et al, 1966).

IgE is produced by the plasma and B-cells. Its normal plasma concentration is very low, approximately 2.4 ng/ml. IgE is involved in allergic reactions especially in the respiratory tract (Dodig et al, 2008). IgE has a half-life of approximately 10 days (Winter, 2000).
QUESTIONNAIRES

An inexpensive way to gather data from a large number of respondents is to use a “questionnaire”. Self-report is an acceptable method to investigate several disorders including DM, cancer and cardiovascular disease, and their associated risk factors (Buhlin et al, 2002; Pitiphat et al, 2002; Blicher et al, 2005, Quan et al, 2008). A well-designed questionnaire is able to gather information on both the overall performance of the test system as well as information on specific system components. Questionnaires are also useful to evaluate the oral health attitudes of individuals (Dagli et al, 2008). Validity and reliability of questionnaires suggest the quality of collected data (Belia et al, 2003; Masood Mirza et al, 2007).
CANDIDA ALBICANS

Yeasts are conventional constituents of the standard oral flora in humans; conversely, they are also recognized as opportunistic pathogens. Candida (C.) albicans, the most common yeast species in the human oral cavity, and several related Candida species are strategic pathogenic fungi, which reside in the oral cavities of healthy humans without causing detrimental effects (Kleinegger et al, 1996). The innate and acquired host defense mechanisms act in concert with the resident bacterial flora, such that Candida grows and survives as commensals. Although, secretory IgA is a major defender of the oral mucosa, the inhabitant microbiota still persists in the oral cavity. Survival of native oral bacteria may be due to their ability to neglect the immune system or perhaps, over a period of evolutionary adaptation, they have achieved a symbiotic state with the host (Marcotte and Lavoie, 1998). Aspartic proteinase, produced by C. albicans can hydrolyze almost all immunoglobulins, including IgA, and several host defence anti-fungal proteins, such as salivary lactoferrin (Schaller et al, 2005). Other Candidal species, including C. dublinensis, C. tropicalis and C. parapsilosis have also shown similar proteolytic activity (Schaller et al, 2005).

Figure 5. Oral Candida colonized on Sabauraud’s dextrose agar plate
The virulence of *Candida* species, especially *C. albicans*, increases in immunocompromized subjects. Immunosuppression provides an excellent platform for fungal proliferation. Diseases where the immune system is suppressed include DM, liver cirrhosis, acquired immune deficiency syndrome and nutritional deficiencies (Pereira et al, 2004). Xersotomia or dry mouth, a common complaint in diabetic subjects predisposes the oral mucosa to opportunistic infections by microorganisms, especially *C. albicans* (Rees, 1994). It has been documented that patients with DM harbor high levels of *C. albicans* in their oral mucosa while intensive Candidal colonization can be seen in diabetic patients with elevated glycemic levels (Belazi et al, 2005).

Oral Candidal colonization has also been associated with smoking habit (Rindum et al, 1994). A possible explanation that has been given in this context is that *C. albicans* utilizes aromatic hydrocarbons, such as those found in tobacco smoke, for nutrition which facilitates their proliferation (Soysa and Ellepola, 2005). Studies have shown that there is no significant difference between growth of oral Candida species and betel-quid/AN chewers and non-chewers (de Miranda et al, 1996; Reichart et al, 2005). *C. albicans* has also been isolated from the oral cavities of chewers of smokeless-tobacco (Bethke and Reichart, 2004).
HABITS AFFECTING PERIODONTAL HEALTH

- Smoking

The unpleasant effects of smoking on general health have been markedly documented (Boyle, 2005; McGhee et al, 2005). Nicotine is an important chemical in tobacco smoke, which causes serious destructive effects on human health (Hukkanen et al, 2005). Smoking is an inevitable risk factor for oral inflammatory conditions and has a prevalence of 16.1% in Pakistan (Nasir and Rehan, 2001). It has been shown that smokers have higher plaque index (PI) as well as an increased number of periodontal pockets (4mm< 6mm) compared with non-smokers (Javed et al, 2007). The number of missing teeth (MT) and MBL are also higher in smokers compared with non-smoking subjects (Javed et al, 2007). Smoking tends to mask the clinical signs of inflammation (BOP) by suppressing gingival bleeding (Hunter et al, 2008). Compared to non-smokers, the reduced BOP in smokers has been associated with constriction of peripheral blood vessels and an increase in epithelial thickness caused by nicotine (Villar and de Lima, 2003; Nakamura et al, 2005; Lichtenberg and Ingemarsson-Matzen, 2005). The mechanism by which tobacco smoke enhances periodontal inflammation is still unclear. However, tobacco smoke is recognized as an essential exogenous source of AGEs (Peppa and Vlassara, 2005). A recent experimental study has shown that there is an increased expression of receptor of AGEs in gingival tissues of smokers, which may be related with periodontal conditions associated with smoking (Katz et al, 2007).

Decreased levels of IgG have been reported in the saliva of smokers compared to non-smokers (Norhagen and Engström, 1998). Studies have also demonstrated that gingival bleeding (GB) may dramatically increase after cessation of smoking habit (Nair et al, 2003). It has been documented that salivary IgA levels rapidly decrease in subjects who have stopped smoking for one week but they recover to their normal concentration two weeks after termination of their tobacco smoking habit (Griesel & Germishuys, 1999). Tobacco smokers harbor significantly high numbers of oral \textit{C. albicans} colonies compared with non-smokers (Soysa and Ellepola, 2005). It is suggested that aromatic hydrocarbons found in tobacco smoke, act as nutritional factors for \textit{C. albicans} and facilitate their proliferation (Soysa and Ellepola, 2005).
Areca nut chewing

The harmful effects of AN on oral soft tissue have been discussed (Trivedy et al, 2002). AN (Figure 6) is a fruit of Areca Catechu and is a major constituent in betel-quid (paan). AN use is popular in South-East Asian countries including Pakistan, India, Bangladesh, Sri Lanka, Malaysia and Thailand (Gupta and Ray, 2004; Gupta and Warnakulasuriya, 2002). It is known as Supari in Urdu. A high prevalence of AN chewing (74%) has been reported among children in Karachi, Pakistan (Shah et al, 2002). Individuals with AN chewing habit experience more GB compared with smokers and non-tobacco users (Amarasena et al, 2003). It has also been shown that AN extracts suppress the growth and protein synthesis of human periodontal fibroblasts (Petro and Zhang, 1997; Chang et al, 1998).

![Figure 6. Areca nuts](image)

The long term use of AN has been associated with fibrosis of oral soft tissues, which may lead to a difficulty in mouth opening (Ariyawardana et al, 2006). It has also been shown that arecoline plays a vital role in the pathogenesis of oral submucous fibrosis and oral cancer (Chang et al, 2001; Kiran Kumar et al, 2007).
Betel-quid chewing

Betel-quid, locally known as *paan*, tends to refer to the glossy heart-shaped betel leaf (*Piper betel*), which is not botanically related to the betel nut palm (*Areca catechu*). A *quid* has been defined as "a substance, or mixture of substances, placed in the mouth or chewed, which remain in contact with the mucosa, usually containing one or both of the two basic ingredients, tobacco and/or AN, in raw or any manufactured or processed form" (Zain et al, 1999). Figure 7 (a) shows betel-quid with tobacco and Figure 7 (b) shows a betel-quid without tobacco.

![Betel-quid with tobacco](image1) ![Betel-quid without tobacco](image2)

**Figure 7.** Betel-quid (a) with tobacco and (b) without tobacco

Betel-quid use is common in South-East Asian countries, including Pakistan, India, Bangladesh, Sri Lanka, Malaysia and Thailand (Gupta and Ray, 2004; Gupta and Warnakulasuriya, 2002). Betel-quid chewers commonly add tobacco to the quid and smoking habits are also common in these subjects (Gupta and Ray, 2004). A recent study has shown that periodontal inflammation and number of MT are significantly higher in betel-quid chewers compared with non-chewers (Akhter et al, 2007). Betel-quid chewers may also harbor higher levels of infection with *A. actinomycetemcomitans* and *P. gingivalis* than non-betel-quid chewers (Ling et al, 2001).

In Pakistan, 6.4% of medical students use smokeless tobacco out of which 2% are habitual betel-quid chewers (Imam et al, 2007). Other forms of smokeless tobacco being used by students include *gutka* and *niswar* (moist snuff). The prevalence of smokeless tobacco use in Karachi has been reported to be 16.1% (Rozi and Akhtar, 2007).
Gutka chewing

Gutka, a dry, relatively non-perishable mixture of tobacco, AN, slaked lime (aqueous calcium hydroxide) and sandalwood fragrance (Gupta and Ray, 2004), has become the most socially acceptable form of tobacco consumption in Pakistan, and is fast replacing the more traditional betel-quid or paan. According to an Indo-Asian News Service Report, approximately 45,000 kg of gutka is sold in Karachi, Pakistan on a daily basis. Unlike cigarettes, gutka comes without a health warning and lack of awareness of its impact on health increases its consumption.

Figure 8. Gutka.

Gutka is initially placed in between the maxillary and mandibular teeth and lightly chewed. It is then held against the buccal mucosa over a long period of time and continued to be gently chewed and sucked intermittently. The constituents may either be spat out or swallowed when desired. A study compared the nicotine content in smokeless tobacco from India, Sweden and the United States, where gutka, smokeless tobacco from India, was shown to have the highest concentration of nicotine (Figure 9) (McNeill et al, 2006).
In South-East Asia, regular gutka use is associated with several deleterious health consequences, most significantly oral cancer (Changrani and Gany, 2005). However, in some Western countries, such as Denmark, smokeless tobacco consumption is not commonly associated with cancer (Holmstrup and Pindborg, 1988). It has also been shown that AN extracts suppresses the growth and protein synthesis of human periodontal fibroblasts (Petro and Zhang, 1997; Chang et al, 1998). Smokeless tobacco contributes to sub-epithelial inflammation and plays a part in tissue destruction (Chang et al, 1998; Holmstrup and Pindborg, 1988). These in vitro results suggest that gutka, which contains AN as well as tobacco as chief components, use may play a role in gingival and periodontal inflammation. Slaked lime due to its high alkaline pH may be associated with oral mucosal inflammation (Nair et al, 1990).

A longitudinal study showed that high consumption of smokeless tobacco is associated with metabolic syndromes, although, these individuals also had a deprived SES, which has a significant association with metabolic disorders, including DM (Norberg et al, 2006, Javed et al, 2007). However, it has also been reported that there is no association between snus consumption and the prevalence of T2D (Eliasson et al, 2004). Gutka has the highest concentration of nicotine compared to other forms of smokeless tobacco as shown in Figure 9.
AIMS OF THE THESIS

GENERAL AIM

The general aim was to explore the association between periodontal inflammation and DM with reference to clinical and radiological periodontal status, self-reported oral symptoms, oral yeast colonization, salivary protein concentrations and smoking, as well as tobacco-chewing habits.

SPECIFIC AIMS:

- **Study I:**
  To assess the periodontal, as well as socioeconomic status among subjects with and without T2D.

- **Study II:**
  To evaluate the association between self-perceived oral health and salivary proteins in children with T1D.

- **Study III:**
  To investigate the periodontal conditions and oral symptoms in *gutka-chewers* with and without T2D.

- **Study IV:**
  To explore the association between periodontal conditions, oral *C. albicans* colonization and salivary proteins in subjects with T2D.
SUBJECTS AND METHODS

This thesis is based on four studies, referred to in the text by Roman numerals (Study I to Study IV). The data were collected through questionnaires, clinical examinations, radiological investigations and laboratory based techniques.

ETHICAL CONSIDERATIONS

The research project was approved by the regional ethical review board in Stockholm, Sweden and the ethical committee of Altamash Institute of Dental Medicine, Karachi, Pakistan. All subjects gave their written informed consents before inclusion.
Participants (type 2 diabetic and non-diabetic subjects) with ages ranging between 45 to 64 years were included in Studies I, III and IV. These subjects were recruited from Punjab Colony, a residential area of Karachi, Pakistan.

In Study I, the clinical examinations, radiological investigations and measurement of RBGL were performed at a local Oral Healthcare Centre in Karachi, Pakistan.

In Study II, type 1 diabetic children were cached from the Diabetes Care Unit of a Child Healthcare Centre in Karachi, Pakistan. The non-diabetic children (Controls) were randomly recruited from the Punjab colony. The ages of type 1 diabetic and non-diabetic children ranged between 10 to 19 years. Oral symptoms were perceived with a questionnaire. For the children with T1D, measurement of RBGL and collection of UWS samples was performed at the Child Healthcare Centre. For the controls, these investigations were carried out at an Oral Healthcare Centre. SFR was recorded for both groups. The duration, family history, treatment and latest HbA1c levels for children with T1D were obtained from the hospital records.

In Study III, a self-perceived oral health questionnaire was administered to type 2 diabetic and non-diabetic gutka-chewers and non-chewers at a local Oral Healthcare Centre in Karachi, Pakistan. The RBGL for these individuals were also recorded at the same Oral Healthcare Centre.

In Study IV, clinical examinations, and collection of UWS and oral yeast samples were performed at a local Oral Healthcare Centre in Karachi, Pakistan.
PERIODONTAL EXAMINATION

Study I

In Study I, periodontal conditions and marginal bone levels were investigated in subjects with poorly-controlled and well-controlled T2D, and non-diabetic controls. Clinical examination included the measurement of PI (plaque index), BOP and PD (probing pocket depth) (4mm < 6mm and ≥ 6mm) at four sites (mesial, buccal, distal and palatal/lingual) on each tooth, excluding maxillary and mandibular third molars. Number of MT was recorded. MBL in the maxillary and mandibular premolars and molars was digitally measured. In addition, RBGL and SES were recorded for both groups.

Teeth with embedded root remnants were considered as “missing”. Maxillary and mandibular third molars were excluded from the study.

- Dental plaque

  Full-mouth presence or absence of dental plaque (oral biofilm) at the gingival margins, buccal, mesial, distal and lingual/palatal was examined using a hygiene index (%). A binomial approach was adopted for scoring, that is, 0= no plaque, 1=plaque (Ainamo and Bay, 1975).

- Bleeding on probing

  A full-mouth clinical inflammation was evaluated by running a periodontal probe along the soft tissue wall of the gingival crevice. The gingiva adjacent to the teeth, buccal, mesial, distal and lingual/palatal was assessed for inflammation and given a binomial score (0= no BOP, 1= BOP present) (Mühlemann and Son, 1971).
• **Probing pocket depth and number of missing teeth**

PD was also measured at four sites, mesial, distal, buccal, and lingual/palatal on all maxillary and mandibular teeth, excluding the third molars. PD was measured to the nearest millimetre with a graded probe (Hu-Friedy Manufacturing, Chicago, IL). Periodontal pockets of 4 to < 6 mm and ≥ 6 mm and the number of MT were recorded.

• **Radiographs**

Panoramic radiographs (Kodak T-MAT, Eastman Kodak, Rochester, NY, USA) were taken of all the T2D and control individuals. The radiographs were transported to the Department of Oral Radiology, Karolinska Institutet, Huddinge, Sweden, where they were scanned (ScanJet 4C/T, Hewlett Packard, Greeley, CO, USA) and examined on a monitor (Nokia 447PRO, Nokia Display Products, Irving, TX, USA). Calculations were made using a computer program (Image Tool 3.0 Program, Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, TX, USA).
**Study II**

In *Study II*, the participants comprised of children with medically diagnosed T1D and controls (non-diabetic children). Data concerning self-perceived oral symptoms among children with T1D and controls was gathered with a questionnaire. Duration of T1D, family history of the disease and current mode of treatment and the latest HbA$_1c$ levels were obtained from the hospital records. UWS samples were collected. Salivary Ig A, IgG and total protein concentrations were determined using standard techniques. SFR and RBGL were recorded.

**Study III**

In *Study III*, the participants, with and without T2D, were questioned about *gutka* chewing habits and their perceived oral health status. Subjects aged 45-64 years were included. Individuals using *gutka*, with and without T2D, were designated as “*gutka-chewers*”. Subjects who reported to have never used tobacco in any form were categorized as “non-chewers”. Periodontal status (PI, BOP, PD [4mm < 6mm and ≥ 6mm]), MT, oral symptoms, reasons for *gutka* use and RBGL were recorded. Exclusion criteria were smoking and use of antibiotics, non-steroidal anti-inflammatory drugs and steroids.

**Study IV**

*Study IV* is a quantitative study in which UWS and oral yeast samples were collected from individuals with T2D. Yeast isolates and salivary IgA, IgG and total protein concentrations were determined using standard techniques. Clinical examination included the measurement of PI, BOP and PD (4mm < 6mm and ≥ 6mm) on four sites, mesial, buccal, distal and palatal/lingual, on each tooth, excluding maxillary and mandibular third molars. In both groups, the RBGL and SFR were also measured.
COLLECTION OF ORAL YEAST AND UNSTIMULATED WHOLE SALIVA SAMPLES

Oral yeast (Study IV) and UWS (Studies II and IV) samples were collected between 10:00 am and 1:00 pm. Participants were instructed to refrain from eating, smoking and drinking for at least two hours before collection of UWS samples.

The dorsum of the tongue was scrapped to collect Candida samples. Each yeast sample was collected by using a dry and sterile cotton swab (COPAN, Amies Charcoal single swab, CE 0124, Italy). The swabs were returned to the containment tube immediately after sampling. The clinical isolates were identified at Karolinska University Laboratory, Sweden, by cultures on BD Difco Sabouraud’s Dextrose Agar (Becton, Dickinson and Company, Sparks, Maryland) and CHROMagar (CHROMagar, Microbiology, Paris, France) incubated at 35°C for 48 hours followed by 30 °C for 72 hours.

To collect the UWS samples, participants were comfortably seated in a “bent forward” position and instructed to spit for five continuous minutes, without swallowing into a clean plastic funnel connected to a measuring cylinder. The volume of saliva was immediately measured and stored in disposable 3.5ml plastic tubes with lid (Sarstedt, Lot: 4071801, Nümbrecht, Germany) under optimal conditions. After sampling, all frozen samples were transferred to Karolinska University Hospital (Department of Laboratory Medicine, Division of Clinical Immunology) at Huddinge, Sweden.
DETERMINATION OF SALIVARY IgG AND IgA CONCENTRATIONS

The UWS samples were centrifuged at 13,000 g at 4°C for 15 minutes to remove cells and debris. The supernatant was collected and stored at -70°C until further investigation.

Levels of total salivary IgG and IgA were determined by direct enzyme-linked immunosorbent assay as described earlier (Mellanen et al, 2001). Briefly, microtiter plates (Corning Inc. NY, USA) were coated with 100 µl per well of anti-human IgG and anti-human IgA (DAKO A/S, Denmark) in coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6) and incubated at room temperature for 24 hours. After washing, 100 µL/well of appropriately diluted IgG (Human serum protein calibrator DAKO A/S, Denmark) and IgA (human colostrum) standards, positive control (saliva from a healthy subject), negative control (saliva from IgA deficient adult subject) and patient saliva samples were added to the respective microplate wells. After incubation at room temperature, the microplates were washed to remove unbound proteins. Purified alkaline phosphatase conjugated anti-human IgG and IgA (DAKO A/S IgA/AP, Denmark) were added (100 µL/well) and the microplates were incubated for three hours at room temperature. After washing, 100 µL/well of substrate (p-nitrophenyl phosphate) in 1.0M diethanolamine, 0.5 mM magnesium chloride, pH 9.8, (Sigma S-0942) was added.

The absorbance was read at 405nm in a microtiter plate photometer (Molecular Devices, Vmax, Sunnyvale, CA, USA).

DETERMINATION OF SALIVARY TOTAL PROTEIN CONCENTRATION

A protein assay reagent kit (Bicinchoninic Acid, [BCA™] Protein Assay Kit, Product No. 23227, Lot No. FE68924, Pierce Chemical, Co., Rockford, IL, USA) was used to determine the total protein concentration in UWS. Using albumin as the standard, aliquots of patient saliva dilutions (200µL/well) were placed in microtiter plates (Costar 3590 96-well assay plate, Corning Inc. NY, USA). The protein assay reagent was added following which, the plates were incubated at 37°C
for 30 minutes. Optical densities were read at 550 nm in a microtiter plate photometer (Molecular Devices, Vmax, Sunnyvale, CA, USA).

IDENTIFICATION OF ORAL YEAST SAMPLES

Identification to species level was determined by a yeast identification system (API 32-C System BioMérieux Yeast Identification Programme, Lyon, France). If identification was not possible with the API 32 system, the yeast isolate was subjected to molecular identification.

❖ Molecular identification

For deoxyribonucleic acid (DNA) isolation, yeast cells were suspended in 200µl sterile polymerase chain reaction (PCR)-grade water, and genomic DNA was prepared using MagNA Pure (Roche Diagnostics GmbH, Mannheim, Germany), a DNA preparation robot (Knepp et al, 2003). For DNA sequencing and polymerase chain reaction (PCR) analysis, a region, about 500-bp, of 18S ribosomal ribonucleic acid (rRNA) gene was amplified by PCR using universal primers and AmpliTaq Gold DNA polymerase. Primers and free nucleotides from the PCR products were removed using QIAquick PCR Purification Kit (Qiagen, GmbH, Hilden, Germany). The purified PCR products were processed for DNA sequencing with BigDye Terminator Cycle Sequencing using capillary electrophoresis technology in ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Both strands of PCR amplified DNA fragments were sequenced to avoid error of sequencing (Jalal et al, 2000). The DNA sequence was analyzed with DNA software, and used to search matching sequences in the Blast DNA Database for yeast identification and typing (Gharizadeh et al, 2004; Jonasson et al, 2002).
MEASUREMENT OF RANDOM BLOOD GLUCOSE LEVELS

RBGL was measured for the diabetic and non-diabetic individuals using a glucometer (ACCU CHEK, Advantage System/Sensor Comfort Strips, Roche Diagnostics, Mannheim, Germany). It is a practical method to check the RBGL in pre-diagnosed diabetic patients (Lambert and Chapman, 2004).
STATISTICAL METHODS

All statistical analyses were performed using Statistica 7.1 (Statsoft, Inc. 1984-2005, Tulsa, USA). In Studies I, II, III and IV, the Bonferroni adjustment post-hoc test was performed for multiple comparisons. A probability of less than 0.05 was regarded as “significant”.

In Study I, MBL on the mesial and distal aspects of premolars and molars from both arches was used as an independent variable, and was expressed as a mean with a 95% confidence interval. The association between mean MBL as a dependent variable and the variables of DM (yes/no), diabetic status (poorly-controlled/well-controlled), PD, PI, BOP, age, gender, smoking (yes/no), and MT as predictors were assessed using one-way analysis of variance (ANOVA). T2D and smoking were statistically significant predictors.

In Study II, multiple logistic regression was used to determine whether the dependent variables (salivary IgG/mg protein, IgA/mg protein, total protein concentration and self-perceived oral health) were statistically significant with the independent variables. The independent variables were transformed into dichotomous variables, for example, children with T1D = 1 and control children= 0.

In Study III, ANOVA was used to determine whether the dependent variables (PI, BOP, PD [4mm < 6mm and ≥ 6mm]), number of MT and oral symptoms were statistically significant with the independent variables. The independent variables were transformed into dichotomous variables, for example, gutka-chewers in subjects with T2D = 1 versus non-chewers in subjects with T2D =0; gutka-chewers in subjects without T2D = 1 versus non-chewers in subjects without T2D= 0. The presence or absence of oral symptoms, such as GB, bad breath and dry mouth, were dichotomized as “1” and “0” correspondingly.

In Study IV, the significance of differences between the dependent variables, Ig levels, protein concentration, oral Candida colonization, numbers of teeth and periodontal conditions, in individuals with T2D was determined using multiple logistic regression. The independent variables were categorized as dichotomous variables; for example, individuals with T2D = 0 and control individuals = 1.
RESULTS

Study I

A total of 75 individuals with T2D and 99 non-diabetic patients underwent clinical and radiological examinations. These individuals ranged in age from 45 to 64 years. Individuals with poorly-controlled T2D had increased MBL in molars and maxillary premolars compared to individuals with well-controlled T2D. PI, BOP and PD of 4mm to < 6mm were increased in individuals with poorly-controlled T2D compared to those with well-controlled T2D and non-diabetic individuals.

Individuals with T2D had increased number of MT compared with non-diabetic controls (P<0.01). There was no difference in the number of MT in subjects with poorly-controlled and well-controlled T2D.

No variation was detected between the diabetic groups when PD was ≥ 6mm. Individuals with poorly-controlled T2D had a lower SES compared to subjects with well-controlled T2D (P <0.05). In addition, no difference was observed with illiteracy and the number of MT between subjects with poorly-controlled and well-controlled T2D.

Amongst the controls patients, PI, BOP and PD (4mm < 6mm) were significantly higher in the oldest age group (60-64 years) compared with subjects between 45-49 years. There was no association of age with PI, BOP and PD (4mm < 6mm) in subjects with T2D.

In the control group, smokers had a higher PI (P<0.001), PD (4mm < 6mm) (P<0.05) and MBL (P<0.05) compared with non-smokers. BOP was significantly reduced in control smokers compared with non-smokers (P<0.01), but there was no difference between these variables in type 2 diabetic smokers and non-smokers.
Study II

The mean HbA1c levels for children with poorly-controlled and well-controlled T1D were 12.2% (range 11.5-15.2%) and 5.5% (range 5.1-6.2%) correspondingly. The means of RBGL in children with poorly-controlled and well-controlled T1D were 13.5 mmol/L (range 11.7 mmol/L – 14 mmol/L) and 5.8 mmol/L (5.4 mmol/L – 6mmol/L) respectively. In controls, the mean RBGL was 5.3 ± 0.8 mmol/L but ranged between 5.2 mmol/L and 5.9 mmol/L.

Self-perceived GB, bad breath and dry mouth were reported to be higher in diabetic children when compared with those in controls ($P < 0.05$). Bleeding gums were more often reported by children with poorly controlled T1D compared to well-controlled type 1 diabetic ($P < 0.05$) and non-diabetic children ($P < 0.001$). There was no difference in self-rated GB between the controls and children with well-controlled T1D. Bad breath was commonly perceived by subjects with poorly-controlled T1D compared with well-controlled T1D ($P < 0.05$) and non-diabetic children ($P < 0.0001$). There was no difference in self-rated burning sensation in mouth between the type 1 diabetic and control subjects. Children with poorly controlled and well-controlled T1D showed no difference in relation to self-perceived dryness in the oral cavity.

There was a significant difference in SFR between the diabetic (mean 0.2 ml/min, range 0.1-0.4 ml/min) and control (mean 0.5 ml/min, range 0.3-0.7 ml/min) ($P<0.01$) groups. Although, there was no variation in SFR between children with poorly-controlled (mean 0.1 ml/min, range 0.1-0.3ml/min) and well-controlled T1D (mean 0.2 ml/min, range 0.1-0.4 ml/min).

Levels of IgG/mg protein were higher in the diabetics when compared with those in control children ($P < 0.05$). In IgA/mg protein levels in diabetic and control groups, no distinction was detected, and no difference between the levels of IgA per mg protein and total protein concentration between the diabetic groups was apparent. IgG/mg protein levels were raised in children with poorly controlled T1D compared to children with well-controlled T1D ($P < 0.05$).
Study III

Gutka-chewers in subjects with T2D had a higher PI ($P<0.05$), BOP ($P<0.05$), PD ($4\text{mm} < 6\text{mm}$) ($P<0.01$) and PD ($\geq 6\text{mm}$) ($P<0.001$) compared to gutka-chewers in subjects without T2D. Non-chewers in subjects with T2D also had a raised PI ($P<0.01$), BOP ($P<0.01$), PD ($4\text{mm} < 6\text{mm}$) ($P<0.001$) and PD ($\geq 6\text{mm}$) ($P<0.001$) compared to gutka-chewers in subjects without T2D. Non-chewers in subjects with T2D had increased PI ($P<0.01$), BOP ($P<0.01$), PD ($4\text{mm} < 6\text{mm}$) ($P<0.01$) and PD ($\geq 6\text{mm}$) ($P<0.001$) compared to non-chewers in subjects without T2D.

Gutka-chewers in subjects without T2D had more MT (mean 11.2; range 6-14) compared to non-chewers in subjects without T2D (mean 5.2; range 3-10) ($P<0.01$), although, no variation in the number of MT between gutka-chewers and non-chewers in subjects with T2D was found.

GB ($P<0.01$), bad breath ($P<0.01$) and dryness of the mouth ($P<0.0001$) were reported more often by gutka-chewers in subjects with T2D compared to gutka-chewers in subjects without T2D. GB ($P<0.001$) and bad breath ($P<0.001$) were noted on more frequently by non-chewers in subjects with T2D compared to non-chewers in subjects without T2D. Bad breath ($P<0.05$) and dry mouth ($P<0.001$) were described on more occasions by non-chewers in subjects with T2D compared to gutka-chewers in subjects without T2D.

In subjects without T2D, GB was commonly reported by gutka-chewers compared with non-chewers ($P<0.01$) but there was no difference in GB between non-chewers in subjects with T2D and gutka-chewers in subjects without T2D. Furthermore, no alteration in oral symptoms between gutka-chewers and non-chewers in subjects with T2D were detected.

Reasons for why gutka was used, as reported by the subjects with and without T2D, are shown in Figure 10.
Reasons for gutka use

Figure 10. Reasons for gutka use reported by subjects with and without T2D.
In type 2 diabetic individuals, *C. albicans* colonization was significantly increased in males compared with females \( (P<0.01) \).

Type 2 diabetic females had a lower SFR (mean 0.15 ml/min; range 0.1-0.3 ml/min) compared to males with T2D (mean 0.38 ml/min; range 0.2-0.5 ml/min) \( (P<0.05) \). Females with *C. albicans* colonization had higher levels of IgG/mg protein \( (P<0.001) \) and total protein concentration \( (P<0.05) \) compared to males with *C. albicans*. The females also had more teeth (mean number of teeth 19.5; range 13-21 teeth) compared with the males (mean number of teeth 10.2; range 10-16 teeth).

Among type 2 diabetic males and females without *C. albicans* colonization, salivary IgG/mg protein levels were 32.2 µg/mg (range 7-107.7 µg/mg) and 38.6 µg/mg (range 2.8-79.3 µg/mg) correspondingly. In these subjects, the mean salivary IgA/mg protein levels were 463.9 µg/mg (range 155-1489 µg/mg) and 319.8 µg/mg (range 90-1863 µg/mg). Salivary total protein concentrations, in these individuals, were 2181 µg/mg (range 879.5-3812.3 µg/mg) and 3569.4 µg/mg (range 7-107.7 µg/mg) respectively.

Mean number of teeth between type 2 diabetic males and females without *C. albicans* colonization were 12.1 (range 9-15) and 15.6 teeth (range 9-16) respectively.

Type 2 diabetic females with *C. albicans* colonization had higher PI \( (P<0.00001) \), BOP \( (P<0.01) \) and PD (4mm < 6mm) \( (P<0.001) \) compared with males in the same group. Type 2 diabetic males with *C. albicans* colonization had lesser number of teeth (mean number of teeth 10.2; range 12 to 15) compared with females (mean number of teeth 19.5; range 16 to 26) in the same group \( (P<0.0001) \).
CONCLUSIONS AND DISCUSSION

The overall aim of this thesis was to investigate the association between oral inflammatory conditions and DM.

Study I

Study I concluded that individuals with T2D experience increased MBL, BOP and PD (4 mm < 6 mm) compared to non-diabetic controls. An underprivileged SES is a major contributing factor in the progression of periodontal conditions (PI, BOP and PD [4 mm < 6 mm]) in subjects with T2D.

Periodontal conditions and MBL were increased in subjects with poorly-controlled T2D compared to those with well-controlled T2D and non-diabetic individuals. Although, high glycemic levels play a significant role in the pathogenesis of diabetic complications, the involved mechanisms remain unclear. However, the deleterious effects of higher glycemic levels have been associated with the formation of AGEs. These end-products promote periodontal inflammation as well as destruction by reducing the number of fibroblastic and osteoblastic cells that repair the resorbed alveolar bone (Promsudhi et al, 2005; Liu et al, 2006; Taylor and Borgnakke, 2008). Formation of these AGEs has also been associated with advancing age (Taylor and Borgnakke, 2008), and this seems to be an explanation for the increased periodontal conditions in non-diabetic individuals aged 60-64 years compared to non-diabetic subjects aged 45-49 years. The current results showed no association between age and periodontal conditions in subjects with T2D. Since hyperglycemia accelerates the production of AGEs, it may be hypothesized that the pathogenesis of T2D makes the severity of periodontal conditions independent of age. There was no difference in periodontal conditions between subjects with well-controlled T2D and non-diabetic controls, which is in accordance with a recent study (Safkan-Seppälä et al, 2006).

An underprivileged SES is an inevitable risk factor for periodontal inflammation as well as T2D (Norderyd and Hugoson, 1998; Norderyd et al, 1999; Connolly et al, 2000; Borrell et al, 2006). A deprived living standard may compel individuals with DM to neglect their medically prescribed anti-diabetic therapies and seek non-conventional treatments instead. However, such folk remedies may not yield
adequate clinical results. In the present study, all individuals on non-conventional therapy had underprivileged living standards and had RBGL ≥ 11.1 mmol/L. The severity of periodontal conditions (PI, BOP and PD [4 mm < 6 mm]) was also increased in these subjects. Therefore, individuals with diabetes should be educated. Folk remedies, such as herbalism, homeopathy and spiritual treatments, lead to poor glycemic control and increase the severity of periodontal conditions. Studies have also associated deprived living standards with a shorter life expectancy and increased mortality (Marmot et al, 1991; Winkelby & Cubbin, 2003; Brønnum-Hansen & Baadsgaard, 2007).

There is a direct association between periodontal inflammation and tobacco smoking (Norderyd and Hugoson, 1998; Scabbia et al, 2001; Geismar et al, 2006; Anil, 2008; Rosa, 2008). In Pakistan, the prevalence of smoking has been reported to be 16.1% and the habit is dominant amongst males aged between 45 to 64 years (Naisr and Rehan, 2001). In this study, 15.2% of the non-diabetic individuals were smokers (all males). We observed a high PI, PD (4 mm < 6 mm), MBL, number of MT but reduced BOP in non-diabetic smokers compared with non-smokers. Smoking tends to mask the signs of inflammation (BOP) by suppressing GB (Hunter et al, 2008), however, the mechanism by which tobacco smoke enhances periodontal destruction is as yet unclear. It has also been reported that smokers have an increased epithelial base thickness, which may also contribute to the reduction of clinical inflammatory signs in the periodontal tissues (Villar and de Lima, 2003). Tobacco smoke is recognized as an essential exogenous source of AGEs (Peppa and Vlassara, 2005) and a recent experimental study has shown that there is an increased expression of receptor of AGEs in gingival tissues of smokers, which may be related with periodontal conditions associated with smoking (Katz et al, 2007).
Study II

Study II concluded that self-perceived GB as well as salivary IgG/mg protein concentration was higher in children with T1D compared to non-diabetic controls.

Self-report is an effective and accepted way of assessing many population characteristics, risk factors, and diseases (Kentala et al, 2004), however, the manner in which the questions are administered, and the education of the respondents, may influence the reliability of questionnaires. Since children may be unaware of their periodontal health status, a parental questionnaire is normally used to assess the child’s oral health status and a clinical examination is performed to verify the questionnaire (Kentala et al, 2004; Chu et al, 2008). It has been reported that gingival inflammation, which is clinically seen as BOP, is significantly higher in children with T1D compared with non-diabetic controls (Pinson et al, 1995; Lal et al, 2007).

Saliva contains biomarkers of inflammation and may therefore be useful to verify self-perceived GB. We hypothesized that under periodontal inflammatory conditions, self-perceived as GB, levels of salivary IgG are elevated. Therefore, IgG may be a parameter of periodontal inflammation (Sandholm et al, 1987). We observed that self-perceived GB as well as salivary IgG/mg protein concentrations were almost twice as common in children with T1D compared to non-diabetic controls. This may suggest that leakage of the serum-derived IgG into the oral cavity is accelerated as a consequence of gingival inflammation. T1D has been associated with an increased production of inflammatory mediators and hyperglycemia further aggravates the underlying immunological dysfunction (Yalda et al, 1994). The current results showed an increased perception of GB coupled with elevated levels of IgG/mg protein among children with poorly-controlled T1D, compared to children with well-controlled T1D. This reflects the periodontal dysfunction and severity in the inflammatory response.

Inflammation is mirrored in saliva as well as in self-perceived oral symptoms.
**Study III**

This study concluded that in subjects without T2D, *gutka-chewers* have more severe periodontal conditions and oral symptoms compared to non-chewers. On the other hand, in subjects with T2D, the severity of these variables is associated with glycemic levels rather than *gutka* consumption.

Questionnaire studies have shown an association between *gutka* consumption and oral mucosal disorders (Changrani and Gany, 2005). The current results showed that, in subjects without T2D, periodontal conditions (PI, BOP and PD (4 mm < 6 mm) and self-perceived GB were more severe in *gutka-chewers* compared to non-chewers. This severity in periodontal conditions and oral symptoms may be explained by the constituents of *gutka*, which mainly include powdered tobacco, slaked lime (aqueous calcium hydroxide) and AN. It has been shown that smokeless tobacco, besides increasing the blood pressure and heart rate, also increases the gingival blood flow (Mavropoulos et al, 2001). This is most likely to occur as a consequence of the activation of the sensory nerves and release of vasodilatory peptides from their peripheral endings (Mavropoulos et al, 2001). It has been shown that extracts from AN, mainly arecoline, suppress the growth of gingival keratinocytes and periodontal fibroblasts (Jeng et al, 1999). The strong alkaline environment in the oral cavity induced by slaked lime may also contribute to oral mucosal inflammation.

In the present study, there was no difference in periodontal conditions as well as oral symptoms between *gutka-chewers* and non-chewers in subjects with T2D. An interesting finding of this study was that even the non-chewers in subjects with T2D had severe periodontal conditions and oral symptoms compared to *gutka-chewers* in non-diabetic individuals. It was notable that the *gutka-chewers* and non-chewers in this group had hyperglycemia, that is, their casual plasma glucose levels were > 11.1 mmol/L. Hyperglycemia has been associated with accumulation of AGEs in periodontal tissues (Liu et al, 2006). These end-products encourage periodontal inflammation as well as destruction (Taylor and Borgnakke, 2008). Therefore, it seems that under hyperglycemic conditions, periodontal inflammation is mainly influenced by T2D and the contribution of *gutka* in this context is rather secondary.
**Study IV**

*Study IV* concluded that females with T2D carrying oral *C. albicans* colonization have increased periodontal conditions and express raised levels of salivary IgG/mg protein and total protein concentration. Type 2 diabetic females carrying oral *C. albicans* have more teeth compared to type 2 diabetic males with oral *C. albicans* colonization.

The results from this study showed a gender difference in the clinical and salivary parameters of periodontal inflammation (BOP and IgG/mg protein). These parameters were elevated particularly in type 2 diabetic females carrying oral *C. albicans* compared to males. Although, the males and females had similar RBGL, the SFR was nearly twice as high in males compared with females. A possible explanation for this finding, is that the size of the salivary glands, is smaller in females compared with males (Inoue et al, 2006). A raised salivary IgG concentration has been reported in patients with DM, which reflects a raised oral inflammation (Anil et al, 1995; Javed et al, 2008). The intensity of periodontal inflammation has been related to the number of teeth affected (Yamanaka et al, 2005). This suggests that a greater number of teeth with inflamed periodontal tissues allow an extensive leakage of IgG into the oral cavity through the gingival crevices. In subjects with *C. albicans* colonization, levels of IgG/mg protein were approximately twice as high in females compared with males. However it was notable that these females had nearly twice as many teeth as males in the same group. Therefore, among type 2 diabetic subjects with *C. albicans* colonization, the presence of more teeth and high gingival blood flow seems to be the most likely explanation for the higher IgG/mg protein levels in females compared with males.

Numerous factors including wearing dentures, xerostomia, age and number of teeth influence oral candidal carriage (Yamanaka et al, 2005; Wang et al, 2006; Ship et al, 2007). The oral health questionnaire, from the present study, revealed that in subjects with *C. albicans* colonization, denture wearing was more frequent in males (47%) compared with females (16.6%). An explanation that has been given in this context is that dentures, either partial or complete, obstruct the salivary flow from minor salivary glands and the free exchange of oxygen. Thus, the resultant low pH level facilitates the growth of *C. albicans* (Aizen et al, 2004).
There is an inverse association between SFR and oral candidal colonization. The current results showed that males, despite having a higher SFR, had increased *C. albicans* colonization compared with females. This may once again be associated with denture-wearing, which was more prevalent in males compared with females.

Albeit, there is a positive relationship between *C. albicans* colonization and age (Ship et al, 2007), the males and females with *C. albicans* colonization had similar mean ages. Therefore, age does not seem to influence the prevalence of *C. albicans* in these individuals.

It has been reported that oral mucosal inflammation is increased in females compared with males and that *Candida* has the ability to colonize the periodontal pockets (Figueiral et al, 2007; Urzua et al, 2008). The present results showed a higher PI, BOP and PD (4 mm < 6 mm) in females with *C. albicans* colonization compared with males. However, it is notable that these females had a lower SFR and had approximately twice as many teeth as males. Therefore, the presence of more teeth coupled with a reduced SFR and its dilution effect might be one of the factors that may have increased the periodontal inflammation, salivary IgG/mg protein and total protein concentration in these females compared with males.
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