Review of Literature

Introduction:

In India, awareness regarding oral hygiene resulting in oral infection is still in its infancy, though today we have around 300, dental colleges including government and private, approximately producing 30000 dentists with 3000 relevant specialist every year. Interestingly, besides traditional tourism, India is now emerging as one of the privileged tourist junction for oro-dental treatment of international standards. From the very beginning several oral diseases, like dental caries, gingivitis, periodontitis, and others remain a challenge for the dental surgeon. In addition, though the oral cavity/mouth is always a major and continuous source of pathogenic microbes, the improper maintenance of oral hygiene worsens other systemic conditions which the patient is already suffering.

Among several systemic diseases, it has been well established that the risk factor for the development of periodontitis is always high in poorly controlled diabetic patients (Albert et al., 2006; Tsai et al., 2002; Loe,1993). Nevertheless, the same studies also predict about the adverse affect of glycemic control in diabetic patients, suffering from periodontal infection, which ultimately indicates the bidirectional linkage between the two conditions. The exact linkage temperament is not yet recognized, though the data on periodontal disease therapy for poorly controlled diabetics indicate the overall reduction in patients’ insulin requirements, and improved glycemic control along with other metabolic balance.
In spite of poor glycemic control, diabetics are seen not at all concerned with the pathogenicity of the microflora of periodontitis patients, but by other various medical factors (Oliver and Tervonen, 1994; Yuan et al., 2001; Thorstensson et al., 1995; Farge, 1992; Bjelland et al., 2002). From the very beginning, diabetes mellitus and periodontitis are two very common chronic diseases, which are somehow considered to be biologically linked.

Current evidence regarding this link supports diabetes and persisting hyperglycemia leading to an exaggerated immuno-inflammatory response to the periodontal pathogenic bacteria challenge (Southerland et al., 2005; Nishimura and Murayama, 2001), resulting in rapid periodontal tissue destruction in manifolds. However, to apprehend the proper linkage between these two diseases, we must shed some light on the basic concept of the same.

**Diabetes mellitus:**

Diabetes mellitus is a metabolic disorder caused mainly due to the defect in either the secretion or the total agility of Insulin. In addition to causing chronic hyperglycemia it also disturbs the carbohydrate, fat and protein metabolism. Diabetes occurs due to disorder of endocrine system where body is not able to utilize the glucose consumed with the diet or produced by the liver. It can be diagnosed by assessing glycated Hb Levels. Periodontitis is considered as sixth complication of Diabetes (Loe, 1993). Medically there are 3-4 types of physiological complications which come under diabetes mellitus, but the periodontitis patients mostly get affected by the occurrence of following two types.

i) The Insulin dependent Diabetes mellitus (IDDM) is medically referred as Type 1 DM (diabetes mellitus) under which the patient suffers from the deficiency of insulin mainly due to the autoimmune destruction of B-cells of islets of Langerhans of pancreas.

ii) Type 2 Diabetes mellitus is as also known as Non- Insulin Dependent Diabetes mellitus (NIDDM). In this situation, the body develops resistance to Insulin due to inability of pancreas. It occurs due to the inadequate secretion of insulin or production of more than normal level of insulin; as under these conditions, the body cells fail to recognize the insulin due to dampen of the relevant receptors.

**Periodontitis:**

Periodontitis is one of the most serious consequences of infections of the oral cavity that affects the protective and supportive tissues around the tooth. In fact, the oral cavity harbours’ numerous bacteria that live in complete harmony with the human host. These
indigenous bacteria indeed colonize in the teeth, tongue, gingival, palates, tonsils etc. as they absorb nutrients and get favourable habitat for their growth in the same. These bacteria usually becomes pathogenic whenever an imbalance occurs inside the oral cavity and cause infections in like dental caries, plaque, resulting into inflammation like periodontitis, gingivitis etc. Periodontitis is a polybacterial infection under which the patient losses the periodontal supporting structures like gingival, cementum, periodontal ligament alveolar bone etc. It generally develops from biofilm infections of the gingival sulcus at the interface of the gingival tissues and the tooth. In the initial state, the sulcus transforms into a pathologically deepened pocket throughout the oral cavity. Finally, a delicate balance is struck between the bacteria and the host’s immune system, resulting in the destructions. If it is left untreated it may lead to tooth loss.

Numerous studies reported a higher prevalence of periodontal disease among diabetic patients than among healthy controls (Firatli, 1997). A positive relationship between poor glycemic control in persons with type 2 diabetes mellitus and increased periodontitis is always being an evidenced. However, the literature also provides consistent evidence of greater prevalence and severity of periodontal disease in diabetics, both types 1 and 2. As per a report forwarded by the International Diabetes Federation (IDF) in India 9.2% of the population belonging to the age group of 20-79 years old are suffering from diabetes which is only second to China. In the present scenario, the total number of diabetics is 90 million (National Diabetes Data Group, 1985), which may rise up to 130 million by 2030, if proper step would not be taken. Further, among Indians diabetes is also realized to begin quite earlier in life, and for the same chronic long-term complications is solely responsible.

**Complications arouse due to Diabetes in Periodontitic patients:**

It is universally accepted that diabetes is usually associated with high and progressive oral complications i.e., gingivitis, periodontitis, periapical abscesses and alveolar bone loss. Due to long standing hyperglycemia in diabetics, the damaging of kidneys, eyes, nerves, blood vessels, and heart are well reported. In addition, the most prevalent and important complication about buccal alterations, such as periodontal disease has also been evidenced with various other alterations that can appear before and sometimes predispose to periodontal disease. The mechanism initiate with dampened salivary gland which ultimately reduce the salivary secretion and alters its composition, tastelessness, coated tongue, burning mouth leading to high susceptibility for buccal infections, slow healing process, decays, halitosis, low production rate of gustin and zinc deficiency (Negrato and Tarzia, 2010; Mese and Matsuo, 2007; Shatzman and Henkin, 1981). Diabetes is also fatal for gum diseases where it reduces the saliva content, which otherwise controls the bacterial growth and continuously washes the sticky foods and prevent the
In longitudinal survey, the risk of progressive bone loss and attachment loss over time were evidenced in diabetics (Taylor et al., 1998). In diabetics the function of immune cells i.e., neutrophils, monocytes and macrophages usually metamorphose. During the same, neutrophilic adherence, chemotaxis and phagocytosis are altered suppressing the defense against bacteria in the periodontal pouch, which ultimately elevate the destruction of the periodontal membrane (Manouchehr-Pour et al., 1981). Proliferation of such pathogens ultimately facilitates the soft tissue deterioration. Large and deep pockets usually develop in the periodontitis patients where bacteria continuously grow, infect and inflame the host tissue. Further, for periodontium, the Polymorphonuclear leukocytes (PMNs) always act as the primary defence cells. Unfortunately, in poorly controlled diabetes abnormalities in PMN functions are evidenced which makes the host more susceptible to infections (Díaz-Romero and Ovadía, 2007).

Up to far extent the implication process by which diabetes influences the periodontium is alike to the patho-physiology of the classic microvascular and macrovascular diabetic complications. Thus, the death rate from diabetic nephropathy was recorded to be the 8.5 times higher in those with severe periodontitis (Mealey et al., 2007). Though, the inception of gingival inflammation and subsequent destruction of periodontal tissues are always by bacterial biofilm (Socransky and Haffajee, 2005), but its alone concurrence is only 20-30% of the variance in disease expression (Grossi et al., 1994). In other words, the bacterial biofilm alone is insufficient to explain disease initiation and progression. As per Offenbacher (1996) the periodontal tissues destruction is mainly due to the host’s inflammatory response to the bacterial challenge. Besides other factors, diabetes mellitus are well characterized to modify the host response to the bacterial challenge and increases the risk for periodontal disease at any stage of life.

Complications arouse due to Periodontitis in Diabetic patients:

Due to severe panic condition, proper chewing of food is always a difficult task for the patient suffering from periodontitis (including loss of dental attachment) as a result the patient remains in under fed condition. Further, due to total periodontal bone loss and the sensitivity of the alveolar mucosa diabetic patients faces much difficulty to hold properly the false dentures.

However, the periodontal diseases are highly infectious in nature, until date various researches have been carried out to establish the basic differences in the subgingival microbial flora of patients with and without diabetes (Mealey and Ocampo, 2006). Till date, 700 bacterial species capable of colonizing the mouth have been characterized from various studies and the number competes absolutely with the flora found in the
human colon (Díaz-Romero and Ovadia, 2007). Bacterial infections always decrease the efficacy of insulin receptors on target tissue cells, which ultimately devoid one’s ability for proper utilization of glucose. Destructive metabolic control of diabetes elevates manifold the risk of developing diabetic complications (Mealey, 1999). In one of our study, we found 11 different colonies of bacterial cultures common in the oral cavities of healthy subject, Diabetic patient without periodontitis and Diabetic patient suffering from periodontitis. Surprisingly, we found bacterial colonies were 35% more dense in the diabetic subjects suffering from periodontitis while compared to diabetics without periodontitis and which is just double than the population recorded from the healthy subjects (Kulshrestha et al., 2011). Inflammatory consequences always initiate and propagate due to the effect of bacterial products viz., endotoxin and/or lipopolysaccharide (LPS). In addition to the inflammatory response, chronic hyperglycemia also creates complications in diabetes mellitus. The periodontal pathogens along with their product usually introduce into the systemic circulation of the periodontitic patients and contribute to immune responses which ultimately exert adverse effects on the homeostasis of the circulatory and immune systems (Gurav and Jadhav, 2011). This situation also deteriorates the glycemic control in diabetic patients.

**Clinical linkage between Periodontitis and Diabetes mellitus:**

Periodontitis and diabetes are two common cosmopolitan diseases having high ubiquity. It has already been accepted by the dental/oral physicians that some relationship exists between these two diseases. Reports are also available suggesting that periodontal treatment may enhance the metabolic control of diabetes. Recent studies are concentrating much on identification of possible mechanisms that underlie these associations and consequently whether the treatment of oral diseases anyhow leads to an improvement in markers of systemic disease. It is well established that the degree of glycemic control is an important factor in the relationship between diabetes and periodontal diseases.

Periodontal diseases are the chronic systemic inflammatory type (Hujoel et al., 2001) and the respective relevant gram-negative infections elevate the insulin resistance and exert negative impact on glycemic control of the patients (AAP, 1996). To weaken the periodontal inflammation, though the periodontal therapy has been found to affect the insulin resistance and glycemic control, but the exact molecular mechanism is still not understood. It is well established that the local as well as systemic dissemination of inflammatory mediators, mainly the cytokines TNF-α and IL-6, usually increases insulin resistance. Further IL-6 itself acts to stimulate TNF-α production, which ultimately lead to additional insulin resistance.
While stimulated by periodontal pathogens, leading to higher systemic cytokine levels, the monocyte of diabetic patients are capable to produce up to 32 times more TNF-α as compared to the non-diabetic patients (Salvi et al., 1997). Further, in diabetic patients in contrast to down-regulated neutrophil activity, monocytes and macrophages are seen to be always hyper-responsive to periodontal infection, resulting in increased production of pro-inflammatory cytokines, especially IL-1α, TNF-α, and PGE 2 (Salvi et al., 1997a,b; Offenbacher, 1996). Potent bacterial products in the periodontal lesion, such as *P. gingivalis* endotoxin, enhance TNF-α production significantly more in peripheral blood monocytes from diabetic patients than in monocytes from non-diabetic patients (Salvi et al., 1997). Abnormalities have also been evidenced with poorly controlled diabetes with their Polymorphonuclear leukocytes (PMNs) function, which are indeed the primary defense cells of the periodontium. This condition dampens the tissue physiology like adherence, chemotaxis and phagocytosis, collectively construe the host more susceptible to infections. Interestingly, abnormalities in PMN function can be markedly improved with insulin therapy and meticulous control of the disease.

Hyperglycemia due to long-term elevation of blood glucose concentrations is always the major complication in diabetics, due to which the formation of a compound “advanced glycation end-products” (AGEs) generates. These AGEs act to “prime” endothelial cells and monocytes, making them more susceptible to stimuli that induce the cells to produce inflammatory mediators. These AGEs targets mainly to endothelial cells and monocytes, making them more susceptible to stimuli that induce the cells to produce inflammatory mediators. Higher concentration of AGEs in the plasma and tissues of diabetic patients create diabetic complications. Few reports indicate that the AGE-enriched gingival tissue has greater vascular permeability, responsible for greater breakdown of collagen fibers and shows accelerated destruction of both non-mineralized connective tissue and bone (Lalla and D’Ambrosio, 2001). Tissue collagenase is found in an active form in diabetic patients, whereas the same remain in the latent form in non-diabetic patients (Ryan et al., 1999). In highly diabetic patients, collagen becomes cross-linked and looses the soluble property (Sorsa et al., 1992). Thus, the AGE remains attached to proteins till the lifespan of those proteins and even if hyperglycemia is cured, the AGE remains intact and never returns to normal position. The AGE thus formed accumulates in the periodontium, causing changes in the cells and extracellular matrix (ECM) components. Collagen produced by fibroblasts under these conditions is susceptible to rapid degradation by matrix metalloproteinase (MMP) enzymes, such as collagenase, the production of which is significantly higher in diabetes mellitus (Monnier et al., 1996; Golub et al., 1998).

The interrelationships between periodontitis and diabetes provide an example of systemic disease predisposing to oral infection, and once that infection is established,
the oral infection exacerbates systemic disease. In this case, it may also be possible for the oral infection to predispose to systemic disease. In order to understand the cellular/molecular mechanisms responsible for such a cyclical association, one must identify common physiological changes associated with diabetes and periodontitis that produce a synergy when the conditions coexist. A potential mechanistic link involves the broad axis of inflammation, specifically immune cell phenotype, serum lipid levels and tissue homeostasis. Diabetes induced changes in immune cell function produce an inflammatory immune cell phenotype (up-regulation of pro-inflammatory cytokines from monocytes/polymorphonuclear leukocytes and down regulation of growth factors from macrophages). This predisposes to chronic inflammation, progressive tissue breakdown and diminished tissue repair capacity. Periodontal tissues frequently manifest these changes because they are constantly wounded by substances emanating from bacterial biofilms. Diabetic patients are prone to elevated low density lipoprotein cholesterol and triglycerides (LDL/TRG) even when blood glucose levels are well controlled. This is significant, as recent studies demonstrate that hyperlipidemia may be one of the factors associated with diabetes-induced immune cell alterations. Recent human studies have established a relationship between high serum lipid levels and periodontitis. Some evidence now suggests that periodontitis itself may lead to elevated LDL/TRG. Periodontitis-induced bacteremia/endotoxemia has been shown to cause elevations of serum proinflammatory cytokines such as interleukin-1 beta (IL-1 beta) and tumor necrosis factor-alpha (TNF-alpha), which have been demonstrated to produce alterations in lipid metabolism leading to hyperlipidemia. Within this context, periodontitis may contribute to elevated proinflammatory cytokines/serum lipids and potentially to systemic disease arising from chronic hyperlipidemia and/or increased inflammatory mediators. These cytokines can produce an insulin resistance syndrome similar to that observed in diabetes and initiate destruction of pancreatic beta cells leading to development of diabetes. Thus, there is potential for periodontitis to exacerbate diabetes-induced hyperlipidemia, immune cell alterations, and diminished tissue repair capacity. It may also be possible for chronic periodontitis to induce diabetes.

Conclusively, Periodontitis disease is highly ubiquitous and insidious, it communicates with pulp tissues through many channels or pathways. Perhaps these channels also get involved in extending pulpal infections to the periodontium and vice versa (Meshack et al., 2011). Thus, it has become the earliest necessity of the time for the health care professionals to understand all the risks factors before going for any substantial treatment. The proper and timely diagnosis for periodontitis is always the first challenge, as during the initial stage the disease is quite painless and patient rarely seeks care. The further challenges include properly controlling all such factors contributing to this particular disease. The removal/attrition of bacterial plaque and calculus in the subgingival
environment by scaling and root planning (hand instruments or ultrasonic devices) are the most successful management resources for periodontitis patients (Shaddox and Walker, 2010; Kaldahl et al., 1996). However, the most important challenge in the treatment of chronic periodontitis is to maintain the periodontium for long term, medically referred as supportive periodontal therapy, or periodontal maintenance. Several physiological and biochemical related medical factors of the patient have to be considered to take any final decisions regarding retreatment, when indicated. However, the disease is highly ingenuous, dentists must be aware of other local and systemic medical factors that could contribute to the disease process and healing response (Shaddox and Walker, 2010). Reports are available indicating that mechanical periodontal treatment not only improves periodontal health, but has an effect on the level of glycosylated hemoglobin.

As it has already been discussed that till date the number of studies found a higher ubiquity of periodontal disease among diabetic patients than among healthy controls (Firatli, 1997), a complete glycemic index of the periodontitic patient must be available with the relevant physician before treatment. Several studies of diabetic subjects with periodontitis have shown improvements in glycemic control following scaling and root planning combined with adjunctive systemic doxycycline therapy (Grossi et al., 1996, 1997; Miller et al., 1992). Further, the meager wound healing is one of the most common complications of diabetes mellitus, the collagen synthesis, maturation, and general turnover also get highly affected. In a glucose-rich environment, the reparative capacity of periodontal tissues is dampened (Salvi et al., 1997b) by the reduction of collagen and glycosaminoglycans which are the major structural protein in the periodontium.

**Other Infectious Diseases in Dentistry:**

The human oral cavity contains a number of different habitats, including the teeth, gingival sulcus, tongue, cheeks, hard and soft palates and tonsils, which are colonized by bacteria. The presence of nutrients, epithelial debris, and secretions makes the mouth a favorable habitat for a great variety of bacteria. The presence of microorganisms on hard and soft oral tissues is fully compatible with health. Dental plaque, dental caries, gingivitis and periodontal disease result from actions initiated and carried out by the normal bacterial flora. Oral bacteria include Streptococci, Lactobacilli, Staphylococci and Corynebacteria, with a great number of anaerobes, especially Bacteroides (Mager et al., 2003; Evaldson et al., 1982).

The indigenous microbiota plays an important role in health and diseases of humans and animals. Indigenous bacteria can survive in the oral cavity because they are less susceptible to or can avoid immune mechanisms (McCulloch, 1993; Kolenbrander et al., 1996). It contributes to the development of the immune system and provides resistance to colonization by pathogenic micro-organisms. It also constitutes a reservoir of potentially
pathogenic bacteria that may infect host tissues (Kolenbrander et al., 1996; Mane 2008). In the oral cavity, indigenous bacteria are often associated with the etiology of two major oral diseases, Dental caries and Periodontitis, which are endemic in industrialized societies and are increasing in developing countries. Oral diseases seem to appear after an imbalance among the indigenous microbiota, leading to the emergence of potentially pathogenic bacteria (Newman, 1988; Socransky et al., 1988).

Dental infections has been recognized in three typical forms:

i) Dental plaques: It is a naturally constructed biofilm of bacteria, sometimes reaches the thickness of 300-500 cells on the teeth.

ii) Dental caries: It is the destruction of enamel, dentin or cement of teeth due to bacterial activities. It initiates by demineralization of the enamel of teeth due to Lactic-acid bacteria. Actinomyces sp., Streptococcus mutans and various proteolytic bacteria are commonly found in human caries as secondary invaders, contributing to the progression of the lesion.

iii) Periodontal diseases: Bacterial infections that affect the supporting structure of the teeth (gingival, cementum, periodontal membrane and alveolar bone) are referred to as periodontal diseases. The endotoxins, hydrolytic enzymes and toxic bacterial metabolites are involved in this disease. Gingivitis, an inflammatory condition of gum, is the most common form of periodontal disease. Serious forms of periodontal disease that affect the periodontal membrane and alveolar bone may results in tooth loss. Streptococci, Actinomycetes, Spirochetes and Bacteroides are the possible bacteria responsible for the disease.

Oral Microflora:

More than 700 bacterial phylotypes have been reported from oral sites and estimates suggest that any individual harbors around 100-200 phylotypes (Kolenbrander et al., 1996). The microorganisms found in the human oral cavity have been referred to as the oral microflora, oral microbiota, or more recently as the oral micro biome. The term micro biome was coined by Joshua Lederberg “to signify the ecological community of commensals, symbiotic and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease” (Lederberg and McCray, 2001). The mouth presents a succession of different ecological situations with age, and this corresponds with changes in the composition of the normal flora. At birth, the oral cavity is composed solely of the soft tissues of the lips, cheeks, tongue and palate, which are kept moist by the secretions of the salivary glands. At birth the oral cavity is sterile but rapidly becomes colonized from the environment, particularly from
the mother in the first feeding (Socransky et al., 1988; Rotimi and Duerden, 1981). The creation of the gingival crevice area (supporting structures of the teeth) increases the habitat for the variety of anaerobic species. (Evaldson et al., 1982; Kolenbrander et al., 1996). The complexity of the oral flora continues to increase with time and Bacteroides and Spirochetes colonize around puberty (Slots, 1976; Socransky, 1988). The normal bacterial flora of the oral cavity clearly benefits their host who provides nutrients and habitat. The normal flora occupies available colonization sites which makes it more difficult for other microorganisms (non-indigenous species) to become established. Also, the oral flora contributes to host nutrition through the synthesis of vitamins and they contribute to immunity by inducing low levels of circulating and secretory antibodies that may cross react with pathogens. Finally, the oral bacteria exert microbial antagonism against non-indigenous species by production of inhibitory substances such as fatty acids, peroxides and bacteriocins (Newman, 1998; Socransky and Haffajee, 1992; Tanner et al., 1979; Slots, 1976; Genco and Krygier, 1972). Microorganisms from the oral cavity have been shown to cause a number of oral infectious diseases, including caries (tooth decay), periodontitis (gum disease), endodontic (root canal) infections, alveolar osteitis (dry socket), and tonsillitis. Evidence is accumulating which links oral bacteria to a number of systemic diseases, including cardiovascular disease, stroke, preterm birth, diabetes, and pneumonia (Evaldson et al., 1982; Lederberg and McCray, 2001; Genco and Krygier, 1972; Beck and Offenbacher, 2005; Awano et al., 2008).

**Key characteristics of Common bacteria flora found associated with Periodontitis:**

**Agregatibacter actinomycetemcomitans**

*A. actinomycetemcomitans* are small, short, straight or curved rod with rounded ends. It is non-motile and gram negative. It can be cultivated in Trypticase Soy Bacitracin Vancomycin Agar Media where it shows star shaped internal structure. It is Benzidine, Phosphotase, Maltose, Mannitol, Xylose positive, Oxidase negative and can decompose Hydrogen Peroxide (Uematsu and Hoshino, 1992; Ljiljana et al., 2008; Slots, 1982; Mandell, 1984; Riggio et al., 1996).

**Porphyromonas gingivalis**

*Porphyromonas gingivalis* are short coccus to rods (cocco-bacilli). It is non-motile obligate anaerobe and gram negative. It can be grown in Blood Agar, TSBV agar Media and Robertson cooked meat media broth where it shows dark, proteolytic colony and
proteolytic activity respectively (Uematsu and Hoshino, 1992; Kuramitsu et al., 1995; Anderson et al., 1995; Griffen et al., 1998; Ljiljana et al., 2008).

**Prevotella sps**

*Prevotella sps* are short round ended rods. They are gram negative bacteria and can be grown anaerobically in Blood Agar and TSBV agar media showing brown-black pigmented colonies. They are Sacchrolytic, Bile sensitive, Lipase, Indole, Gelatin, Glucose and Sucrose positive and Lactose negative (Uematsu and Hoshino, 1992; Ljiljana et al., 2008).

**Campylobacter sps.**

*Campylobacters* are motile having polar flagellum and are gram negative. They show dark grey stain when grown in anaerobic media (TSBV) and incubated anaerobically (Uematsu and Hoshino, 1992; Ljiljana et al., 2008).

**Fusobacterium sps.**

They are characterised by having cigar shaped cells with pointed ends. They are nonmotile, non-sporing. They are gram negative grows well under anaerobic atmosphere in Blood Agar and TSBV agar media. They are Indole positive, Bile positive, shows Gelatin liquefaction, Esculin hydrolysis negative (Uematsu and Hoshino, 1992; Bennett and Deurden, 1985; Love et al., 1980; Ljiljana et al., 2008).

**Parvimonas micros**

It is gram positive cocci arranged singly or in short chains and is obligate anaerobe so can be grown anaerobically in TSBV agar media. They are Glucose, Maltose positive and Indole, Sucrose, Xylose, Lactose, Coagulase and Nitrate positive (Murdoch et al., 1988; Rams et al., 2012; Ljiljana et al., 2008).

**Eubacterium sps**

It is a gram positive, obligate anaerobic small pleomorphic rod. They are Glucose positive, Indole positive, shows Esculin hydrolysis but negative for Tween -80 and Arginine (Uematsu and Hoshino, 1992; Ljiljana et al., 2008).

**Streptococcus mutans**

They are important in the initiation of dental caries because its activities lead to colonization of the tooth surfaces, plaque formation, and localized demineralization of
tooth enamel. It can be cultivated on Salivarius Mitis agar media (Gold’s Media). It is Mannitol, Raffinose, Sorbitol positive, shows Esculin hydrolysis but is negative for Starch (Aldred et al., 1986; Aaltonen et al., 1987; Uematsu and Hoshino, 1992; Ljiljana et al., 2008).

*Lactobacillus acidophilus*

They are proteolytic bacteria, commonly found in human carious dentin and cementum, which suggests that they are secondary invaders that contribute to the progression of the lesions. They are cultivated in Ragosa SL Agar media and are lactose positive (Ljiljana et al., 2008).

*Actinomycetes*

They are gram positive shows branching and beaded appearance. They are grown well on Blood Agar and are Indole negative (Ljiljana et al., 2008).

**Role of Immune system to overcome the diseases:**

Inside our body there is an amazing protection mechanism called the immune system. It is designed to defend us against millions of bacteria, microbes, viruses, toxins and parasites. When an animal dies, its immune system (along with everything else) shuts down. In a matter of hours, the body is invaded by all sorts of bacteria, microbes, parasites; otherwise none of these things are able to get in when your immune system is working. Once an animal die it only takes a few weeks for afore-state organisms to decay completely its body and utilize it as a part of their nutrition.

The goal of any pathogen depends on its ability to elude host immune responses. Thus, pathogens developed several ways to infect their host, by evading detection or destruction the immune system (Finlay and McFadden, 2006). The animal body represents an ideal habitat to the bacteria, fungi, viruses and other parasites by providing its warmth, moisture, cells and rich supplies of nutrients for their growth. To survive efficiently an animal has to defend from the invasion of micro-organisms (Tizard, 2004). Immune system has been evolved in every animal to protect them from intracellular and extracellular pathogens. This highly specific immune system is the fundamental to survival for a human host (Ivan et al., 2006). Indeed, the Immune system, itself is a biological structures and processes within an organism which protects against diseases. To be effective, an immune system must be able to detect a wide variety of agents, from viruses to parasitic worms, and distinguish them from the organism’s own healthy tissues (Beck et al., 1996).
To recognize and neutralize the wide variety of pathogens which are able to evolve and adapt rapidly and there by suppress detection and neutralization by the immune system, multiple defense mechanisms have also been recognized. These mechanisms include phagocytosis, antimicrobial peptides called defensins, and the process such as complement system (Litman et al., 2005; Mayer and Gene, 2006). A remarkable feature of the immune system is that to possess memory and the ability to remember the previous encounters. This process of acquired immunity is the basis of vaccination (Plotkin, 2005).

In 1890, Behring and Kitasato demonstrated the presence of a protein that played active role in protection, which was named as antibodies. An immunoglobulin is one of them, commonly known as an antibody (Ab), abbreviated as Ig. Immunoglobulin(s) are glycoprotein molecules secreted by plasma cells i.e., the type the white blood cells. The name of immunoglobulin was based on the fact that when antibody containing serum is placed in an electrical field they are found to migrate with globular proteins resultant to immunoglobulin. These are produced by plasma cells in response to an antigen or an immunogen and works as antibodies (Pier et al., 2004). Immunoglobulin plays a very critical and very important part in the immune response by specifically recognizing and binding to particular antigens, such as bacteria or viruses and aiding in destruction of these antigens (Borghesi and Milcarek, 2006). Basically it is a large Y-shaped protein structure produced by B cells and is used by the host immune system to identify and neutralize foreign objects such as bacteria and viruses (Roux, 1999; Al-Lazikani et al., 1997).

Antigen binding is the primary function of antibodies and can result in protection of the host. These antibodies occur in two physical forms, one form is that which is secreted from the cell is called a soluble, and another form that is attached to the surface of a B cell is called the membrane-bound form and is referred to as the B cell receptor (BCR). Once the B cells gets activated then they get differentiated either into plasma cells called as antibody producing cells that secrete soluble antibody or into the memory cells that long live in the body for years afterwards in order to allow the immune system to remember an antigen and respond promptly upon future exposures (Chen et al., 2009). Once an antibody is produced against any specific antigen, its whole process remains imprinted in the memory of the immune system so that whenever in the future if the same antigen enters the body; the immune system follow up the same process and produces more of the same antibodies. Thus the follow-up of the presence for any specific immunoglobulins in the blood can be helpful in diagnosing or ruling out infections or certain other illnesses. Doctors also rely on the immunoglobulin test as one of the tools to diagnose immuno-deficiencies related diseases. Immunoglobulin levels are also used as
part of an evaluation for autoimmune conditions such as rheumatoid arthritis, lupus, and celiac disease (Bailey and Scotts, 2007).

At the time of birth the antibodies are provided by passive immunization from the mother to baby via placenta. After birth within the first years of life endogenous antibody production varies for different kinds of antigen which the host encounters. As these antibodies are present freely in the bloodstream, they become the member of the humoral immune system. There are three ways in which antibodies contribute to immunity: i) by preventing the pathogens from entering or damaging cells by binding to them; ii) by stimulating the removal of pathogens through macrophages and other cells by coating the pathogen; iii) by triggering the destruction of pathogens by stimulating other immune responses such as the complement pathway (Putnam et al., 1979).

Mattu et al., (1998) explained the role of natural antibodies and stated that human beings also produce “natural antibodies” which are present in serum without any previous infection, vaccination, other foreign antigen exposure or passive immunization. These antibodies can activate the classical complement pathway leading to lysis of enveloped virus particles long before the adaptive immune response is activated. Bacteria present in the human gut produce disaccharide galactose α(1,3)-galactose (α-Gal), which is found as a terminal sugar on glycosylated cell surface protein; these antibodies are generated in response to production of this sugar (Milland and Sandrin, 2006; Racaniello, 2009).

The immunoglobulins has been further divided into five different classes, based on differences in the amino acid sequences in the constant region of the heavy chains. All immunoglobulins within a given class have very similar heavy chain constant regions. These differences can be detected by sequence studies or more commonly by serological means, i.e. by the use of antibodies directed to these differences (Mian et al., 1991).

1. IgG - Gamma heavy chains- these are found in all body fluids and are the smallest but most common (75% to 80%) of all the antibodies in the body. IgG antibodies plays a very important role to overcome the bacterial and viral infections. IgG antibodies are the only type of antibody that can cross the placenta in a pregnant woman to help /protect her baby (foetus). Its normal mean serum concentration is 1240 mg/dl. IgG antibody levels if clinically measured would be considered as the indicative of an individual’s immune status to particular pathogens (Azeredo et al., 2002). Thus, measurement of immunoglobulin G could be easily acted as a diagnostic tool for certain conditions (Azeredo et al., 2002; Cox et al., 2008).

2. IgM - Mu heavy chains - IgM antibodies are the largest antibody. They are found in blood and lymph fluid and are the first type of antibody form in response to any infection. IgM also helps other immune system cells to destroy foreign substances. IgM are about 5% to 10% of all the antibodies in the body. Its normal
PhD. dissertation by Reena Kulshrestha

mean serum concentration is 120 mg/dl. IgM antibodies appear during the primary immune response in an early course of an infection. This property of IgM makes it useful in the diagnosis of infectious diseases. Thus demonstrating IgM antibodies in a patient’s serum indicates recent infection, or in a neonate’s serum indicates intrauterine infection (Houghton Mifflin Company, 2004). IgM is also referred as “natural antibody” as in normal serum it is often found to bind to specific antigens, even in the absence of prior immunization (Houghton Mifflin Company, 2004; Erik et al., 1998; Wellek et al., 1976).

3. IgA - Alpha heavy chains- found in sensitive areas of the body such as the nose-breathing passages, digestive tract, ears, eyes and vagina. IgA antibodies protect such body surfaces which are directly exposed to external foreign substances. Thus, this types of antibody is usually abundant in saliva, tears, and blood. About 10% to 15% of the antibodies present in the body are IgA. There are various severe health problems which are only due to the low levels of Immunoglobulin A in the body. It is also because the bacteria produce an enzyme leading to the splicing of IgA antibodies into the Fc and Fab fragment. The most prevalent antibody deficiency is the selective IgA deficiency (sIgA D). Alterations in IgA1/IgA2 ratio very often go hand to hand with specific disease states such as recurring infections of the airways or a kidney disorder called IgA nephropathy.

4. IgD - Delta heavy chains- IgD antibodies are found in small amounts in the tissues that line the belly or chest.

5. IgE - Epsilon heavy chain- IgE antibodies are found in the lungs, skin, and mucous membranes. They cause the body to react against foreign substances such as pollen, fungus spores and animal dander. They may occur in allergic reactions to milk, some medicines, and also in some poisons. IgE antibody levels are often high in people suffering from various types of allergies.

Pishdad and Faghiri, (1995), Rodríguez-Segade et al., (1991) found Immunoglobulins G, A, and M significantly higher in diabetic patients while compared to healthy controls. In the initial investigation, they found higher levels of IgG, IgA and IgM in type I diabetics in comparison to those obtained from healthy controls. They concluded the possibility of immuno-inflammatory abnormality might be the underlying cause for the elevated immunoglobulins in the patients with IDDM (Insulin Dependent Diabetes Mellitus) and its complications.

Further, the hyperglobulinemia seen in both in type I and type 2 diabetes mellitus suggests that the shared metabolic disturbance (chronic hyperglycemia) was the underlying cause for the same finding and that Hyperimmunoglobulinemia could also be the possible
complication of diabetes mellitus (Pishdad and Faghiri, 1995). In their investigation of immunoglobulin levels the diabetics have revealed higher levels of IgG, IgA and IgM while compared to healthy controls. In a subsequent study on a larger group of diabetics, when means of blood sugars were replaced by HgA1c levels, similar results were demonstrated. Therefore it could be concluded that perhaps hyperimmunoglobulinemia is seen in both types of idiopathic diabetes mellitus, the magnitude of chronic hyperglycemia probably does not influence its degree.

The IgG and IgA level in the tissues of both diabetic and non-diabetic subjects suffering from periodontitis were found to be significantly higher than that of healthy subjects (Sukumaran, 2006). Nevertheless, the diabetic patients revealed significantly higher IgG and IgA levels compared to that of the non-diabetic group suffering from periodontitis. On the basis of a study, Anil (2006) concluded that the humoral immune response plays an important role in the pathogenesis of periodontal disease in diabetics in which possibly the significant higher levels of immunoglobulin in the gingival tissues acts as a protective mechanism against the increased bacterial challenges. Elevated antibody levels may explain why poorly controlled diabetes exacerbates periodontal disease (Awartani, 2010).

Charles et al., (1989) performed a check for serum immunoglobulin (G, A, M) levels on patients with non-insulin-dependent (type II) diabetes mellitus (NIDDM) and compared with normal controls and concluded that there was no significant difference between the mean IgG and IgM levels. However, he conceived the higher IgA levels were in the diabetic group when compared with control groups. He added that this rise in IgA levels were true regardless of age, sex, duration of disease, and type of treatment but there were no significant differences in immunoglobulin levels between insulin-treated and non-insulin-treated diabetic groups. The data presented in this study clearly demonstrates that non-insulin-dependent diabetic patients have an isolated elevation of IgA among the immunoglobulins G, A, and M. A number of studies have indicated that cell-mediated immunity is suppressed in the diabetic patient and with the increase in the number of bacterial infections present in diabetic patients, it can be concluded that humoral immunity is also abnormal. While measuring quantitatively in the non-insulin-dependent diabetic patients, IgG and IgM levels were normal and their IgA levels were elevated as compared to the normal control groups (Charles et al., 1989).

Various works have been done to fetch various pathways through which diabetes affects periodontal status but very bleak success has been recorded regarding the impact of periodontal diseases on the diabetes-related inflammatory status (Tunes et al., 2010). As per the conclusion of the work, a wide-ranging activation of the innate immune system causing chronic low-grade inflammation is closely involved not only in the pathogenesis of type 2 diabetes mellitus and its complications, but also in the pathogenesis
of periodontal diseases, whereby cytokines play a central role in the host’s response to the periodontal biofilm. However, till date various reviews attempt to explain the immunobiological connection between periodontal diseases and type 2 diabetes mellitus, exploring the mechanisms through which periodontal infection can contribute to the low-grade general inflammation associated with diabetes and discussing the impact of periodontal treatment on glycemic control in people living with both diabetes and periodontal disease. The subgingival plaque sampled from deep periodontal pockets of periodontitis patients with NIDDM was determined for predominant cultivable microflora. Indirect immunofluorescence for the pathogens; Bacteroides intermedius, Bacteroides gingivalis, and Aggregatibacter actinomycetemcomitans was used to examine these samples. Serum antibody was measured by Enzyme-Linked Immuno-Sorbent Assays (ELISA) (Zambon et al., 1988).

Objective of the Present Studies:

The complete work of the present dissertation work have been carried out by taking three different objectives under consideration.

Besides the Review of literature, in the first part we have compare the micro flora existing in the diabetic and non-diabetic patients suffering from periodontitis. Further, the four most common pathogenic bacteria were chosen and antimicrobial susceptibility test were revealed by taking three different groups antimicrobial agents against them; 1 allopathic drugs ii) potential herbal extracts iii) herbal toothpastes available in the Indian market.

Simultaneously in the 2nd part, the Candida sps. were also isolated from diabetic and non-diabetic patients suffering from periodontitis and the antimycotic susceptibility tests have been performed against the isolated major pathogenic Candida sps.

Further, in the 3rd part, we have compared the serum Immunoglobulin in the Diabetic and non-diabetic patients having Periodontitis. Four different parameters were taken here under consideration; glycated hemoglobin, immunoglobulin G, immunoglobulin A and immunoglobulin M.

To conduct this part we selected four different groups:

Group A: Diabetic patients suffering from Periodontitis
Group B: Diabetic patients not suffering from Periodontitis
Group C: Non-Diabetic patients suffering from Periodontitis
Group D: Non-Diabetic patients not suffering from Periodontitis (Control/Healthy Persons).

Finally we have concluded our complete work on the basis of our obtained results.