Review of literature

This review includes the following items:

A. **Diabetes mellitus; an overview**

B. **Histological changes innormal pulp**

C. **Histological changes of periodontium and teethin diabetic patients**

A.Diabetes mellitus; an overview:

Willis et al (1996) announced that people with type1 diabetes often suffer from great fluctuations in blood glucose level, and usually present with diabetic ketoacidosis.

Dorner et al (1997) mentioned that there is no preventive measure against type1 diabetes, which causes approximately 10% of diabetes mellitus cases in North America and Europe. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type1 diabetes can affect children or adults but was traditionally termed "juvenile diabetes" because it represents a majority of the diabetes cases in children.

Mealey (1998) declared that type2 diabetes mellitus were resulted from insulin resistance, a condition in which cells fail to use insulin properly, formerly referred to as non-insulin dependent diabetes mellitus (NIDDM) or (adult-onset diabetes). At this stage hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver or stimulate the beta cells of islets of Langerhans to increase insulin secretion.

Bergman and Ader(2000) reported that most patients with type2 diabetes mellitus are obese or may have an increased percentage of body fat distributed predominantly in the abdominal region. Adipose tissue plays an important role in the development of insulin resistance. Elevated circulating levels of free fatty acids derived from adipocytes have been demonstrated in numerous insulin resistance states. Free fatty acids

contribute to insulin resistance by inhibiting glucose uptake, glycogen synthesis, and by increasing hepatic glucose production by glycolysis.

Davidson et al (2000) announced that women at high risk are those older than 25 years of age, with positive family history of diabetes, previous personal history of gestational diabetes mellitus or marked obesity.

Rajesh and Joseph (2001) mentioned that most cases of diabetes mellitus fall into three broad categories: type1, type2 and gestational diabetes. A few other types are congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes. Alsorevealed that diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger).

Jean et al (2002) reported thatobesity and type2 diabetes are associated with insulin resistance, the mechanisms of which remain poorly understood. A significant correlation between circulating IL-6 level and insulin sensitivity has recently been found in humans. Because adipose tissue could be a significant source of IL-6, they analyzed the relationship between the levels of adipose tissue IL-6 and insulin action in vivo, during a hyperinsulin-emicnormoglycemic clamp, and in vitro by

measuring glucose transport in adipocytes from 12 obese subjects with (n = 7) or without (n = 5) diabetes. They observed an inverse correlation between adipose tissue IL-6 content and maximal insulin-responsiveness. In conclusion, they mentioned that increased IL-6 production by adipose cells might participate to the insulin-resistant state observed in human obesity.

Manfredi (2004) mentioned that diabetes mellitus (DM) is a group of complex multisystem metabolic disorders characterized by a relative or absolute insufficiency of insulin secretion and/or concomitant resistance to the metabolic action of insulin on target tissues. They announced that chronic hyperglycemia of diabetes is associated with long-term systemic dysfunction.

Saydah et al (2005) declared thatgestaional diabetes occurs when pregnant women, who have never had diabetes before, have a high blood glucose level during pregnancy. It may precede development of type 2 DM. They announced that it resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. They found that it occurs in about 2%–5% of all pregnancies in USA and may improve or disappear after delivery. They confirmed that gestational diabetes is fully treatable but requires careful medical supervision throughout the pregnancy.

Rother (2007) reported that type1 diabetes results from the body's failure to produce insulin, and requires the person to inject insulin, (also referred to as insulin-dependentdiabetesmellitus (IDDM), or (juvenile

diabetes). He found that this type of diabetes is characterized by loss of the insulin-producing beta cell of islets of Langerhans in the pancreas that leads to insulin deficiency. He mentioned that this type of diabetes can be further classified as immune-mediated or idiopathic, as the majority of type1 diabetes is of immune-mediated nature, where beta cell loss is a T-cell mediated autoimmune attack.

Chen et al (2012)observed that type 2 diabetes mellitus (T2DM) and prediabetes had increased among children, adolescents and younger adults. They mentioned that the causes of the epidemic of T2DM are embedded in a very complex group of genetic and epigenetic systems interacting within an equally complex societal framework that determines behavior and environmental influences. They announced that in the past few years considerable emphasis has been placed on the effect of the intrauterine environment in the epidemic of T2DM, particularly in the early onset of T2DM and obesity. They mentioned that prevention of T2DM is a 'whole-of-life' task and requires an integrated approach operating from the origin of the disease.

Janardhan and Sastry (2014)studied dipeptidyl peptidase IV (DPP4) as a promising target for the treatment of chronic metabolic type 2 diabetes mellitus (T2D). They mentioned that DPP4 is a highly specific serine protease involved in the regulation and cleavage of two incretin hormones, glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). These incretin hormones are released by the gastrointestinal tract in response to ingestion of food and stimulate

insulin secretion and thereby regulate glucose homeostasis with a low risk of hypoglycemia and glucagon secretion.

B. Histological changes innormal pulp:

Morse (1991) announced that fibrous tissue accumulation is the beginning of calcification. It occurs to the point where almost nothing exists except the fibrous tissue. This is termed fibrous degeneration or pulp atrophy. He mentioned that it is different from fibrous replacement (such as the replacement of infarcted heart muscle tissue) where the fibrous connective tissue contains viable fibroblasts.

Ranjitkar et al (2002) studied radiographically the incidence of pulp stones in different gender and found that the prevalence of pulp stones was similar in males and females. Comprising 123 males and 94 females aged between 17-35 years, 3296 teeth were examined under 2× magnification on bitewing radiographs. Pulp stones were scored as present or absent, and associations with sex, tooth type, dental arch, side and dental status noted. Pulp stones were found in 100 (46.1 %) of the subjects and 333 (10.1 %) of the teeth examined. Occurrences were rare in premolars (0.4 %) but significantly higher in molars (19.7 %). Pulp stones were significantly more common in first molars than in second molars, also in maxillary first molars than in mandibular first molars. They mentioned that carious and/or restored maxillary right first molars and maxillary left second molars displayed higher prevalence of pulp stones than unrestored and intact molars.

Pashley and Liewehr (2006) histologically recognized two types of stones: those that are round or ovoid, with smooth surfaces and concentric laminations; and those that assume no particular shape, lack laminations and have rough surfaces.

Dukoff (2009) studied age-related changes that affect the pulpal chamber and the canals. He said that noticing age-related changes, such as the narrowing of the pulpal space, is important during treatment. The calcifying process reduces the size of the pulpal chamber and narrows the canal. He mentioned that if total obliteration of pulpal tissue ensues, it hinders the practitioner's treatment of the symptomatic tooth while providing patient care. In his study he found that in many times, the patient was asymptomatic while the chamber and canal were calcifying. He declared that the practitioner may be unaware that the tooth is undergoing age-related changes that would result in a necrotic tooth with a periapical rarefaction in the future. He concluded that calcifying pulpal chambers signal that age-related pulpal changes are taking place in the dental pulp tissue.

Wilson (2011) declared that dental pulp, or root canal system of a tooth, is a living tissue with a blood supply and nerves and millions of odontoblasts. This tissue is responsible for laying down dentin. Over life secondary dentin makes the pulp smaller, and this can easily be observed on X-rays. He mentioned that pulps have a delicate blood supply and are very prone to getting infected if overly traumatized by cavities, injury or severe gum disease.

Sisman et al (2012) carried out a retrospective study to determine the prevalence of pulp stones, possible associations between pulp stones and gender, tooth type and dental arch in a Turkish population. They studied (469) patients having total of 6,926 bitewing radiographs.The mean age was 24(± 10.7). Pulp stones were detected in (10.56%) of examined teeth in females and (4.44 %) in males with significant difference between the genders ($p<0.05$). They found that the occurrence of pulp stones was higher in the maxilla than in the mandible in each tooth type and when data for both arches were combined ($p<0.001$). They found that there were no statistically significant differences between the right and the left side in each tooth type and arch ($p=0.101$). Pulp stones were found in only 96 (9.07 %) of the 3538 premolars and in 962 (90.92 %) of the 3424 molars examined, with differences in occurrence being statistically significant ($p<0.001$).

Satheeshkumar et al (2013) examined 227 patients with age range from 15 to 70 years. Teeth were examined under digital panoramic radiograph. The presence or absence of pulp stones was recorded. The frequency of occurrence of pulp stones with sex, tooth type, dental arches, and types were compared with the types of calcification. The prevalence of pulp stone was found to be higher in the molars in both the arches. Most number of pulp stones reported at the third and fourth decade of life.

Manolea et al (2014) studied normal and inflamed human dental pulp specimens through immunohistochemical and an electron microscope in order to evaluate the morphological aspects of the nerve structures from the dental pulp. They found that even in acute pulpitis no ultrastructural

changes occured in the nerve fibers but prolonged exposure to noxious factors may lead to changes like nerve sprouting.

C. Histological changes of periodontium and teeth in diabetic patients:

Including:

i. **Gingival changes in diabetic patients**
ii. **Periodontal changes in diabetic patients**
iii. **Inflammatory changes and catabolism**
iv. **Immunity changes and microbial infection**
v. **Vascular, Pulpal and periapical changes**
vi. **Pulpal calcification, stones and role of osteopontin (OPN)**

i.Gingival changes in diabetic patients:

Seppäläet al (1997) studied cellular and vascular changes in gingival connective tissue samples taken from 29 diabetic patients. The results compared with 10 non diabetics. Biopsies were taken from sites with no evident signs of periodontitis, and were processed for light microscopy examination, and the blood vessel area was analyzed using an interactive digital analyzing system. Respective volumetric and numeric densities of cellular components were found in diabetic patients; including fibroblasts, neutrophils, monocytes, macrophages, mast cells, lymphocytes, blast cells and plasma cells. On the other hand they recorded decreased fibroblasts

and collagen fiber density in diabetics. According to their results, they announced that blood vessels were recorded in diabetics having the largest mean area of cross-section, with swollen and proliferated endothelial cells.

Ryan and Kamar (2003) studied andconducted searches to identify published human epidemiologic studies; cross-sectional observations; and longitudinal, cohort, case-control and other studies that describe variables associated with diabetes and periodontal disease. Some animal studies are reported to support human findings and explore mechanisms of action. They announced that people with type 1-diabetes have a greater risk of developing gingivitis. More gingival inflammation and higher gingival bleeding scores were found in children with diabetes than children without diabetes after accounting for plaque scores. They also found that type 2 diabetes is also associated with gingivitis that may be related to glycemic control.

Tesseromatis et al (2009) announced that gingivitis and periodontitis are chronic bacterial diseases of the underlying and surrounding tooth tissues. Diabetes mellitus is responsible for tooth deprivation both by decay and periodontal disease. They made a study aimed to investigate the relationship between the gingival lesions and the microangiopathy changes in streptozotocin-induced diabetes mellitus. Forty male Wistar rats were divided into two groups (control and experimental). Diabetes mellitus was induced by 45 mg/kg streptozotocin. The histological investigation of the marginal gingival and the relevant gingival papilla showed inflammation of the lamina propria and the squamous epithelium

as well as marked thickness of the arteriole in the diabetic group, but no changes were observed in the control group. From their results they suggested a probable application of a routine gingival histological investigation in diabetic patients in order to control the progress of disease complications.

Punit and Sheela (2013) collected gingival smears from 30 male subjects, 20 diagnosed with type 2 diabetes and 10 controls.Ten diabetics were with smoking habit and(n= 10) without history of smoking habit. Healthy subjects with no history of smoking or diabetes served as the control group (n = 10). The smears were stained using Papanicolaou procedure. The cellular (CA) and nuclear areas (NA) were measured using image analysis software. A statistically significant ($P< 0.001$) increase in NA and N: C ratio in smoker diabetic group was observed compared to the non-smoker diabetic group and the control group. The non-smoker diabetic group also showed significant increase. There were significant alterations in the cellular pattern of gingival mucosal cells in a non-smoker diabetic, but the alteration was to a greater extent in smoker diabetics demonstrating a synergistic effect of smoking and diabetes on gingival mucosa.

ii.Periodontal changes in diabetic patients:

Koide et al (1995) studied the effects of recombinant human interleukin-1β (rhIL-1β) on alveolar bone resorptive activity in rats. Continuous administration of rhIL-1β or phosphate-buffered saline (PBS) was given via osmotic pumps for 3, 7 and 14 days to rats with silk ligatures around second maxillary molars. Other animals without ligatures were pumped with phosphate-buffered saline (PBS) or remained

untreated. Only rats receiving rhIL-1β exhibited enhancement of inflammatory cell invasion on days7 and 14. In rats receiving rhIL-1β with ligatures, numerous resorptive lacunae containing multinucleated giant cells (MNGCs) was found.

Taylor (1999) reviewed clinical research that had considered the relationship between treatment of periodontal diseases and improvement in glycemic control in humans. The results revealed that patients who had periodic periodontal treatment were more liable for controlling their blood glucose levels.

Lalla et al (2000) had their experiment on streptozotocin diabetic induced mice by 4 daily intraperitoneal injections and controlled injected by citrate buffer. One month after treatment with streptozotocin or citrate buffer, mice were infected with *P. gingivalis.* Evaluation of the extent of periodontal destruction by measurement of alveolar bone loss was determined. In their results they demonstrated that sustained hyperglycemia in mice inoculated with the periodontal pathogen *P. gingivalis* resulted in significantly increased alveolar bone loss 2 months after infection.

Bezerra et al (2002) examined separately the impact of diabetes on alveolar bone loss and the formation of new bone following resorption. Studies were carried out in a model where a ligature was placed around a molar tooth. The ensuing inflammation, loss of attachment, and bone loss were measured over a seven-day period. It was found out that diabetes

had affected the net bone loss by increasing the rate of bone resorption as well as inhibiting the formation of new bone.

Fouad and Burleson (2003) analyzed 540 teeth that had received non-surgical endodontic treatment and had been followed up for at least 2 years. Among all teeth, no difference in success was found between endodonticaly treated teeth of diabetics versus non diabetics. However, among teeth that initially had preoperative periapical lesions (n=178), non-diabetic patients had a higher likelihood of success than teeth in diabetic patients. They found that patients with diabetes have increased periodontal disease in teeth involved endodontically and have a reduced likelihood of success of endodontic treatment in cases with preoperative periradicular lesions.

Engebretson et al (2004) mentioned that poor glycemic control has been consistently associated with periodontal disease severity. Their study aimed to determine whether glycemic control was related to gingival crevicular fluid (GCF) levels of interleukin-1beta (IL-1β). They collected 45 GCF samples from 45 patients with type 2 diabetes and untreated chronic periodontitis. Plaque index (PI), bleeding on probing (BOP), probing depth (PD) and attachment level (AL) were recorded at six sites per tooth. IL-1β levels were determined from individual GCF sample by enzyme-linked immune-absorbent assay (ELISA). Glycated hemoglobin (HbA1c) levels were measured from anticoagulated whole blood using an automated affinity chromatography system. Patients with greater than 8% HbA1c had significantly higher mean GCF IL-1β levels than patients with less than 8% HbA1c. In conclusion they mentioned that poor glycemic

control is associated with elevated GCF IL-1β hence increasing periodontal destruction.

Gokhan et al (2004) compared the thickness of the cementum layer in type 2 diabetic and non-diabetic subjects in order to improve the understanding of dental mobility in type2 diabetes and its effect on tooth loss. A total of 46 male patients with a mean age of 61.72 +/- 5.45 year were included in the study; undecayed single rooted premolar teeth extracted from 46 male patients were used to assess the alterations in the cementum layer in type2 diabetics. Histological preparations from extracted teeth were examined under light microscopy.The average thickness of the cementum layer in the decalcified teeth extracted from non-diabetic patients was compared to the average thickness of the cementum layer in type 2 diabetic patients. A significant difference was observed in every site of measurement between type 2 diabetic and non-diabetic patients with regard to the thickness of the cementum layer. They found that the cementum layer was thicker at the apical part of the root and at the midpoint of the apical half, and thinner at the central part of the root and at the midpoint of the coronal half in type 2 diabetic patients.

Naguib et al (2004) announced that diabetes has been identified as an important risk factor for infection. They made a study that was to investigate how diabetes affects host-bacteria interactions by focusing on the inflammatory response in a connective tissue setting. Diabetic and control mice were inoculated with Porphyromonasgingivalis, a pathogen associated with bite wounds and periodontal disease. The response was measured histologicaly or by the expression of inflammatory cytokines.

By quantitative histologic analysis, there was little difference between the diabetic and control mice on day 1. On day 3, however, the inflammatory infiltrate had subsided in the control group, whereas it had not in the diabetic group ($p<0.05$). Results that were noted at the molecular level by the persistent expression of tumor necrosis factor-alpha (TNF-alpha) and cytokine dysregulation represents a mechanism through which bacteria may induce a more damaging inflammatory response in diabetic individuals.

Graves et al (2005) showed that diabetic mice exhibit prolonged inflammation as a response to infection by P. gingivalis, which is due to TNF dysregulation. This inflammation could also affect the net loss of bone by inhibiting repair of resorbed bone.

Ira et al (2008) reviewed literatures to identify oral conditions that are affected by diabetes mellitus. They also examined the literature concerning periodontitis as a modifier of glycemic control. They resulted that although a number of oral disorders have been associated with diabetes mellitus, the data supported the fact that periodontitis is a complication of diabetes. Patients with long-standing, poorly controlled diabetes are at risk of developing oral candidiasis, and the evidence indicated that periodontitis is a risk factor for poor glycemic control and the development of other clinical complications of diabetes. They announced that evidence suggests that periodontal changes are the first clinical manifestation of diabetes.

Sakallıoğlu et al (2008) declared that diabetes was found to be the causative factor of increasing monocyte chemo-attractant protein-1 in gingival tissue. They investigated and compared the monocyte chemo-attractant protein-1 (MCP-1) levels of gingival tissues in 15 diabetic rats having induced periodontitis with other15 controlled rats. Gingival MCP-1 levels were measured by enzyme-linked immune-sorbent assay (ELISA). Periodontal inflammation was quantified by the inflammatory cell infiltration in the gingival samples, whereas periodontal destruction was assessed by the alveolar bone loss in the experimental regions. This study revealed that MCP-1 concentrations were higher in diabetic group with increased gingival inflammatory cell infiltration and alveolar bone loss.

Rola et al (2010) studied and evaluated the awareness, perception, sources of information, and knowledge of diabetes mellitus and periodontal health among Jordanians, to examine the factors related to their knowledge, and organize effective education programs. A random sample of 500 diabetic patients was recruited from three hospitals and three comprehensive health centers. Completed questionnaires with the answers were filled. Only 28% indicated that they followed up gum diseases with the dentist; 48% were aware that diabetic patients are more prone to gum diseases and oral health complications. About a third (38%) recognized that their periodontal health might affect their glycemic level. The clinical implication of their findings was that dentists, physicians, and other health providers should inspect diabetic patients for gum diseases each time they come for care and recommend that diabetic patients see a dentist regularly.

Ueno et al (2010) studied the periodontal health in diabetic and non-diabetic patients among Japanese population. They announced that the outcome of periodontal disease seemed to be influenced by the diabetic state to some degree, but no clear association between diabetes and oral health status was found.

Ferreira et al (2014) studied the influence of diabetes mellitus at the periapical tissues and the success of endodontic treatment in those patients. They evaluated mobility, fistula, pain on percussion and horizontal and vertical evaluation of final restoration of 37 teeth in the test group and 25 in the control group. They found that regarding the assessment of the success of endodontic treatment, 62% in the test group and 80% in the control group ($p > 0.05$) which was not statistically significant.

iii.Inflammatory changes and catabolism:

Loescheand Grossman (2001) proved that diabetes enhances catabolic activity in periodontal tissue, through activation of some degrading enzymes like matrix metalloproteinase. They reported that periodontal diseases are associated with chronic inflammation. This chronic inflammation leads to production of reactive oxygen species, which in turn activate matrix metalloproteinases (MMPs). These enzymes degrade the collagen in the periodontal ligaments, leading to decreased attachment of the tooth to the alveolar process (which presents clinically as a loose tooth) and deepening of the gingival sulcus.

Catanzaro et al (2006) determined the effect of diabetes mellitus progression on inflammatory and structural components of dental pulp, through making investigations on male Wister rats that were given a single injection of streptozotocin (STZ) to induce diabetes. They declared that nitrite and kallikrein levels in dental pulp tissue were higher in diabetic rats than in controls 30 days after (STZ) injection. Only nitrite was decreased after 90 days from (STZ) injection. The activity of alkaline phosphatase showed significant changes in diabetic rats reaching the highest activity rate in rats after 90 days from date of injection. On the other hand they found that collagen concentration was decreased.

Leite et al (2008) evaluated the function of sialic acid as a hydrogen peroxide scavenger concentration in the dental pulp of diabetic induced rats by streptozotocin (STZ). Moreover other parameters of the antioxidant system, such as peroxidase and catalase activities, of the dental pulp of healthy and diabetic rats were evaluated. They announced that dental pulps of diabetic rats exhibited significantly lower free, conjugated, and total sialic acid concentrations than those of control tissues. They concluded that catalase activity in diabetic dental pulps was significantly enhanced in comparison with that of control pulps, so diabetes could impair responses in the parameters of the antioxidant system of dental pulp tissue.

Mariana et al (2008) evaluated some parameters of antioxidant system of dental pulp of the 4 incisors of 8 healthy and 8 diabetic induced rats by streptozotocin. They reported that catalase activity in diabetic dental pulps was significantly enhanced in comparative with that of

control pulps. In addition reduction of sialic acid could be resultant of reactive oxygen species production.

Stuart et al (2009) determined the effect of hyperglycemia on pulpal healing in their experiment on 22 exposed rat pulps capped with mineral trioxide aggregate. Eleven were streptozotocin diabetic induced rats identified as (DM group) and 11 as (control group). Histologic samples were prepared and evaluated for dentin bridge formation and pulpal inflammation. By analyzing data they found that dentin bridge formation was inhibited in diabetic rats with more inflammation in these pulps.

iv.Immunity changes and microbial infection:

Ueta et al (1993) announced that uncontrolled diabetics of type I and II have more liability to oral infections and periodontitis. They examined the prevalence of diabetes mellitus (DM) in odontogenic infections and oral candidiasis, and influences of DM on the clinical manifestations of the infections and neutrophil functions. Among 21 severe and 221 mild odontogenic infections DM was detected in 5 cases in each group. Of 64 cases of symptomatic oral candidiasis, 8 cases were complicated with DM which was detected by blood examination during treatment. All white blood counts, C-reactive protein levels, erythrocyte sedimentation ratesand odontogenic infection were more elevated in DM. Candidiasis and hyposalivation was also observed. They found that diabetes causes less production of free oxygen radicals and exhibits reduced phagocytosis and intracellular killing of *Candida* cells. Their results indicated that DM is a predisposing condition for odontogenic infections and oral

candidiasis, also DM-complicated infections that become more severe because of neutrophil suppression.

Delamaire et al (1997) studied 61 diabetic patients free of infection (40 Type 1, 21 Type 2), using tests that explore all the functional steps of polymorphneuclear cells (PMN). They found that PMN chemotaxis was significantly lower in diabetic patients than in healthy controls ($p < 0.001$). They concluded that all steps of PMN functioning are altered in diabetic patients, which may increase the risk of vascular complications and infectious episodes.

Tennenberg et al (1999) mentioned that the main cause of increased risk of infection and necrosis of dental pulp of diabetic patients is obliterative endarteritis and poor microvasculature with inhibition of microbicidalpolymorphneuclear (PMN) activity.

Geerlings and Hoepelman (1999) studied the immune dysfunction in patients with diabetes. They resulted that diabetic patients show depressed leukocyte adherence, decreased chemotaxis, phagocytosis and cytokine production, as well as increased adherence of microorganisms to diabetic cells. They mentioned that possibly the carbohydrate composition of the receptor plays a role in this phenomenon.

Iacopino (2001) declared in his collected review article that diabetes mellitus (DM) induces changes in immune cell function and produces an inflammatory immune cell phenotype (up-regulation of pro-inflammatory cytokines from monocytes/ polymorphneuclearleukocytes and down-regulation of growth factors from macrophages). He mentioned that these

changes predispose to chronic inflammation, progressive tissue breakdown, and diminished tissue repair capacity.

Fouad et al (2002) examined the effect of diabetes mellitus or the morbidity and mortality of non- obese diabetic (NOD) mice after inducing periapical lesions with and without inoculations with endodontopathic bacteria. They compared the sensitivities of two microbiological techniques in detecting the persistence of the bacterial inoculum in the mouse molar environment. Acute (1–2 wk) or chronic (5 wk) exposures were either inoculated with a mixture of facultative and anaerobic bacteria or exposed to oral flora without inoculations. After death the teeth in the chronic groups were analyzed for the presence of the inoculated bacteria by culturing. Periapical lesion size was measured histomorphometrically and the interleukin-6 content was measured immunohistochemically. They concluded that the diabetic animals were more susceptible to significant morbidity and mortality, compared with controls, after induction of periapical lesions.

Bender and Bender (2003) declared that diabetics are particularly prone to bacterial or opportunistic infections. They found that this vulnerability is caused by a generalized circulatory disorder whereby the blood vessels are damaged by the accumulation of atheromatous deposits in the tissues of the blood vessels lumen. In addition, blood vessels, particularly capillaries, develop a thickened basement membrane, which impairs a leukotactic response, beside decreasing the polymorphonuclear leucocyte microbicidal ability and failure to deliver the humoral and cellular components of the immune system.

Fernandez and Ricart (2003) announced in their collected review article that periodontal infection may similarly elevate the systemic inflammatory state and exacerbate insulin resistance. Tumor necrosis factor-α, produced by adipocytes, increases insulin resistance by preventing autophosphorylation of the insulin receptor and inhibiting second messenger signaling via inhibition of the enzyme tyrosine kinase. Interleukin-6 is important in stimulating tumor necrosis factor-α. Periodontal infection can induce elevated serumlevels of interleukin-6 and tumor necrosis factor- α, and may play a similar role as obesity in inducing or exacerbating insulin resistance.

Giuliettiet al (2004) carried out an animal study to show the effect of vitamin D on the immune system and inflammation. They announced that in cases of low serum $1.25(OH)_2D_3$ levels and higher glucose concentrations, vitamin D may modulates the pathogenesis of type I diabetes mellitus.

Tunes et al (2010) made a review in attempting 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 (thus aggravating insulin resistance) and discussing the impact of periodontal treatment on glycemic control in people living with both diabetes and periodontal disease. They found that 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, through an ongoing cytokine-

induced acute-phase response, but also in the pathogenesis of periodontal diseases, whereby cytokines play a central role in the host's response to the periodontal biofilm.

Shell et al (2012) declared from their reviewed article that obesity is the hallmark of the metabolic syndrome and predisposes patients to the development of major chronic metabolic diseases including type 2 diabetes mellitus. Adipose tissue expansion in obesity is characterized by increasing infiltration of proinflammatory immune cells causing chronic, low-grade inflammation. Phenotypic switching of macrophages is an important mechanism of adipose tissue inflammation, and there is involvement of cells from the adaptive immune system in this process. Cytokines and chemokines produced by immune cells influence localized and systemic inflammation, which is a pathogenic link between obesity and insulin resistance.

Pishipati (2013) mentioned in his review article that impaired wound healing in DM is because of impaired function of leukocytes as during an inflammatory response, leukocytes adhere to endothelial cells due to the presence of adhesion receptors on leukocytes and endothelial cells. The leukocytes eventually become firmly attached to the vascular wall before migrating into tissues. Accordingly, one possible explanation for the abnormal leukocyte function in DM might be a down regulation of adhesion molecules leading to decreased leukocyte-endothelial cell interactions and a reduced number of leukocytes in inflammatory lesions.

Zhou et al (2013)investigated the effects of type2 diabetes on the subgingival plaque bacterial composition by applying culture-independent 16S rDNA sequencing to periodontal bacteria isolated from four groups of volunteers. A total of 71,373 high-quality sequences were produced from the V1-V3 region of 16S rDNA genes by 454 pyrosequencing. In the subjects with healthy periodontium, the abundances of three genera (Prevotella, Pseudomonas, and Tannerella) and nine operational taxonomic units OTUs were significantly different between diabetic patients and their non-diabetic counterparts. In the subjects carrying periodontitis, the abundances of three phyla (Actinobacteria, Proteobacteria, and Bacteriodetes), two genera (Actinomyces and Aggregatibacter), and six OTUs were also significantly different between diabetics and non-diabetics. They resulted that type 2 diabetes mellitus could alter the bacterial composition in the subgingival plaque.

v.Vascular, Pulpal and periapical changes:

Gafar et al (1989)carried out anatomoclinical study on tissue samples obtained from patients with various chronic general diseases including cardiovascular diseases, diabetes, kidney diseases, liver diseases, acute and chronic leukemias, that had developed before the time when biologic therapy of the dental pulp had been recommended. Vascular changes were found, followed by reactional responses in various stages of trophic changes, or dysplasia in the dental pulp. However they demonstrated that microscopical studies had certain degree of deterioration of the dental pulp, which were counter indicative for biological therapy.

Kohsaka et al (1996) investigated the changes in pulpal and periapical tissues after pulpal exposure in streptozotocin-induced diabetic rats. Control rats were injected with citrate buffer. All animals received a pulpal exposure in the left mandibular first molar. Blood glucose concentration was measured at 0, 7, 14, 28, and 42 days. All animals were killed at 7, 14, 28, and 42 days after pulpal exposure, and their mandibles were evaluated histologically and histometrically. In experimental rats, inflammation in the apical periodontal ligament and root resorption and alveolar bone resorption were more severe than that in control rats. This study also revealed histometrically that, in experimental rats, lesions in the periapical area were significantly larger than those in control rats.

Gordand et al (2003) proved that pulp necrosis, abscess and vaculation seemed to be a successive sequel for long standing diabetes, as he and his assistants examined late changes in the dental pulp of experimental rats with diabetes. The experiment involved 36 male albino rats, initially 35 days old. The animals were separated into 6 equal groups. The first (T1), third (T2) and fifth (T3) groups of animals were given a single dose of alloxantetrahydrate. The second (C1), fourth (C2), and sixth (C3) group of animals were used as appropriate control groups and received 1 ml of physiological saline injection. Body weights and glycemia were measured weekly. The block of mandibular molars was taken for histological examination. The results of histological examinations showed stasis in microcirculation, as well as in large blood vessels of the pulp, necrosis of the pulp tissue found in animals after 63 days of experimental diabetes. Pulps of the animals killed after 95 days showed, besides massive pulp necrosis, abscess forms localized in the

mesial horn of the pulp. They found that pulps of the animals sacrificed after 125 days showed hydropic degeneration of the pulp with massive and diffuse presence of vacuoles in odontoblasts.

Iwama (2003) studied experimental rats. The histological analysis showed that alveolar bone resorption was most severe and the peri-radicular lesions were largest in diabetic rats given the sucrose solution. From their results they suggested that the metabolic conditions produced by type II diabetes enhance the development of periradicular lesions in rats.

Segura-Egea et al (2005) investigatedradiographically the prevalence of apical periodontitis (AP) in patients with and without type 2 diabetes mellitus. Results showed that apical periodontitis in at least one tooth was found in 81.3% of diabetic patients and in 58% of control subjects. Amongst diabetic patients 7% of the teeth had AP, whereas in the control subjects 4% of teeth were affected.

Chen et al (2006) recorded that vascular smooth muscle cells in diabetics show calcifications. They mentioned that high blood glucose level stimulates osteopontin (OPN) production and increases alkaline phosphatase activity, indicating the increasing hard tissue forming function of dental pulp cells.

Bouvet et al (2007) studied diabetes induced by streptozotocin in rats, and revealed that OPN plays a role in the development of diabetic vascular complications as atherosclerosis associated with diabetic duration.

López-López et al (2011) made a cross-sectional study with radiographic records of 50 adult patients reporting a history of well-controlled type 2 diabetes mellitus (DM) (study group) and 50 age- and sex-matched subjects who reported no history of DM (control group). Periapical status of all teeth was assessed using the periapical index score. The results showed that in adult patients, type 2 DM is significantly associated with an increased prevalence of AP of endodontic treated teeth.

Mesgarani et al (2014) evaluated the frequency of periradicular lesions in diabetic patients among Iranian population. A total of 135 diabetic patients were studied. The demographic features as well as the duration of the diagnosis of diabetes (> 48 months was called long term and < 48 months short term) was the quality of control of their diabetes. For all the patients, panoramic and periapical radiography were performed for the presence of any radicular radiolucent lesions. They had done vitality test for the recorded teeth except for the root treated ones, periodontal involvementand necrotic teeth. The frequency of periradicular lesions in long term patients was 85 (94.4%) which was higher than in short term 37 (82.2%) (p=0.023).

vi.Pulpal calcification, stones and role of osteopontin (OPN):

Dilhan (2004) made a radiographic study on 56 insulin dependent diabetic patients and 56 non diabetics. They examined maxillary and mandibular first molars by digitally scanned films. The evaluated teeth were all intact. Nine measurements were made from each image. The results of that study revealed that type I diabetics had more pulp stones

than non-diabetics in maxillary first molars. While there was no significant difference in the presence of pulp stones between type I diabetics and non-diabetics in mandibular first molars. Also no significant difference were found between diabetics and non-diabetics in the crown height, total pulp area, coronal pulp area, area of the clinical crown or heights of the mesial and distal pulp horns.

Goga et al (2008) declared that the correlation to pulp stones has been plethoric as opposed to anemic personalities, metabolic imbalance or dysfunction, orthodontic treatment and traumatic occlusion. Theyfound generalized pulp stones in the dentition of individuals with various conditions. These include dentin dysplasia Type II, familial expansileosteolysis, osteogenesisimperfecta Type I and otodental syndrome.

Inagaki et al (2010) made an experimental study on cultured rat pulp cells, measured the alkaline phosphatase activity and OPN level of pulp cells in glucose and non glucose treated cells. They found a significant increase in both alkaline phosphatase activity and OPN level. In the same report, they announced that OPN might be a key molecule involved in the increase of pathologic pulp calcifications in diabetics, after they had observed immuno-histochemically the increased of OPN protein in rats treated with glucose. Alkaline phosphatase activity in cultured rat dental pulp cells increased in both glucose treated and untreated cells by time, but treatment of cells with glucose significantly elevated the enzyme activity. In 30 and 42 week-old rats, large calcified particles with greater number were present in coronal pulp of diabetic rats than non diabetic

ones. Radicular pulps of diabetic rats form a thickened layer of predentin. They also reported that OPN was found around the calcified particles and in the odontoblast zone under the thickened layer of predentin.

Nayak et al (2010) determined the correlation between pulp stones and cardiovascular disorders, Type II diabetes mellitus, autoimmune disorders and dental wear defects. A total of 1432 teeth of five groups were examined, comprising of patients with C.V.S. disorders; Type II diabetes mellitus, autoimmune disorders, dental wear defects and control group. Teeth were examined under 2X magnification on radiovisiograph (RVG) and conventional intra-oral periapical radiograph. The presence or absence of pulp stones was recorded. Theyfound pulp stones in 134 (9.35%) of 1432 teeth detected. Significantly, higher numbers of pulp stones were recorded in patients with cardiovascular disorder (15.86%) incomparison to typeII diabetes mellitus (7.69%), significantly higher in molars (18.29%) than premolars (6.6%) and in maxillary arch (12.36%) than in mandibular arch (5.95%). On the other hand they found no significant difference between sexes and sides.

Nakajima et al (2013) studied rat dental pulp cells and gingival fibroblasts. Independently they cultured the cells with 50 and 100μg/ml Advanced Glycated End products (AGE). Alkaline phosphatase activity and calcified nodule formation were measured. Expressions of receptor for AGE, osteopontin (OPN), and osteocalcin (OCN) mRNA were determined by quantitative real-time polymerase chain reaction. Protein levels of OPN and OCN secreted in culture medium were quantified by enzyme-linked immunosorbent assay. They found that AGE (50 and 100 μg/mL) markedly increased both alkaline phosphatase activity and calcified nodule

formation in dental pulp cells ($P<0.01$). Real-time polymerase chain reaction analysis revealed that AGE increased mRNA expressions of receptor for AGE, OPN, and OCN in dental pulp cells ($P<0.05$). Enzyme-linked immunosorbent assay analysis revealed that the protein levels of OPN and OCN produced by dental pulp cells were higher in AGE-treated than in untreated cells ($P<0.05$). Soit is revealed that AGE enhanced the calcification potentials of rat dental pulp cells, suggesting that it may stimulate pathologic calcification of diabetic dental pulp tissues.