Discussion

It is generally known that when teeth have a proper endodontic treatment, the success rate will be high. However there are still some systemic diseases (such as diabetes mellitus) which may affect the procedures as well as the outcome of endodontic treatment as it affects the dental pulp, causing many pathological changes such as narrowing of the pulp chamber and canal space which creates a clinical challenge for the practitioner in locating the orifice of the canal space becomes difficult. Also diabetes increases the incidence of pulp stones, these may block the access to the root canal space and the orifices may become recessed. Furthermore, removing all dystrophic calcifications and pulp stones from the pulp chamber will be a tedious process. Yet, removing all of calcifications in the chamber is a prerequisite to removing all pulp tissue. Moreover, negotiating a "tight" canal can be a perilous procedure therefore care must be taken to prevent instrument bending or separation, creation of false canals or perforation.

A prospective study of the histopathological changes of human dental pulp of diabetic patients was done. This kind of study differs from many researches which made their histopathologic examination on rats or cultured cells in which diabetes were induced artificially as that done by **Inagaki et al (2010)**, or made radiographic examination on human teeth as **Dilhan et al (2004)**. Only few researches concerned with studying the histopathology of human dental pulp such as **Bissada and Sharawy (1970)**.

This difference:

1. Gives more realistic results as it based on human being.
2. Gives more accurate results as the early changes of pulp can be detected, and the accurate site, size and shape of stones and calcified particles can be determined.
3. Overcomes the limitation of radiographic examination of disability to notice the early pulpal changes, to give full detailed data about the size and the configuration of pulp stones, as well as the possibility of presence of artifacts. All these limitations do not exist in the present study.

Examination of dental pulps of 45 diabetic patients (group B) and 20 healthy persons (group A) was done. With doing the best in defining the inclusion criteria clearly and exclude all general and local criteria that seemed to affect dental pulp such as cardiovascular disease and periodontal disease respectively. This number of samples is more than that taken in previous researches that worked on human being. They had the limitation of small sized group samples as seven patients that were taken by **Russell (1967)** and 21 taken by **Bissada and Sharawy (1970)**.

Sound teeth were extracted. Adequate pulp fixation has always been a challenge, and artifacts resulting from inadequate fixation are described as evidence of pathosis. We have overcame this problem by widening the apical foramen by fine ended stone before dropping the tooth in 10% buffered formalin solution to assure complete fixation of the internal pulp tissues. We preferred not to cut the apical 2-3mm of the roots as done by

Torabinejad and Kiger (1985) as this technique destroys the apical pulp tissue.

In our study we have made longitudinal sections of dental pulp of 5µm thickness that gave us many advantages:

a. Allowed obvious generalized screening along the tooth from coronal part to apical part.
b. Decreased the number of slides taken for each sample.
c. Decreased the effort needed to examine each tooth without skipping any part.
d. It was less expensive.

However, other authors as **Sheykhrezaee (2007)** claimed that transverse sections of samples reduces the risk of pulp rupture and loss during tissue handling, furthermore allow more possible evaluation of all pulp tissue in a discrete distance from the apex.

Each slide was examined under light microscope to the power 40. Some slides needed to be magnified to the power 100 or 200. According to **Yaltirik et al (2004)** inflammation observed in slides were classified histopathologically into none, mild, moderate and severe. Fibrosis was defined as increased fibroblasts and collagen fibers. Calcification was graded as diffuse, complete and stone existence. According to degree of destruction necrosis was graded as no necrosis, partial or complete. Presence of vaculation or any combination of findings was noticed. If none of the above mentioned findings was seen, dental pulp was considered normal.

Mild inflammation in group A had the least percent in samples (2.2%), while severe inflammation had the highest (20%). But none of

samples showed normal pulp. This might be because the long duration (8-10) years of diabetes. In contrast none of samples in group B revealed any signs of inflammation (0%), (except single excluded case had mild inflammation and replaced by new specimen; to not affect either total number or histopathological findings of control group). **Catanzaro et al (2006)** reached the same results in founding increased pulp infiltration by PMNs and macrophages at 30 days after striptozotocin (STZ) treatment of male Wistar rats.

Areas of calcification and stones were the main concern of the study. Under the power of ×40 the surface area in (μm^2) of each stone was calculated by an image analyzer device, using a software program* in which manual tracing of the outline of each stone was done by mouse pointer. Group A had greater incident percent of pulp calcification (diffuse and complete) and pulp stones (total 51% and 26.6% respectively). This can be considered as the result of chronic hypoxia and cell death. Our finding coincided with **Inagaki et al (2010)** who found large calcified particles in coronal pulp of diabetic rats. Furthermore, they found a thickened layer of predentin in the radicular pulp. In contrast, **Nayak et al (2010)** found the lesser number of pulp stones in Type II diabetes mellitus (7.69%) on the studying the correlation between systemic disorder and pulp stones.

None of samples in control group revealed necrosis, while (26.66%) of diabetic group had partial necrosis. These data explain the role of longer period of diabetes which might have changed pulpal tissues and lead to necrosis. Findings in this study revealed only presence of partial necrosis

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which cannot exclude the possibility of the occurrence of complete necrosis. **Lima et al (2013)** had found necrosis in their results and was explained as pulps from patients with diabetes have the tendency to present limited dental collateral circulation, impaired immune response, increased risk of acquiring pulp infection (especially anaerobic ones), besides occasional tendency towards pulp necrosis caused by ischaemia.

Angiopathy was found in 30/45 specimen (66.6%) in group A including dilated blood vessels, thickened blood vessel wall, ruptured blood vessel or hemorrhage. Blood vessel calcification was seen only in diabetic group with (22.22%) of total specimens emphasizing role of diabetes in pulp angiopathy. **Catanzaro et al (2006)** gave similar results on noticing both generalized accumulation of atheromatous deposit in the blood vessels lumen and increased endothelial cell permeability that impair the leukotactic response and decreased the leukocyte microbicidal activity.

Diabetic group showed statistically significant higher prevalence of hypertensive patients than control group; as none of control group had hypertension. It was known that hypertension and diabetes are correlated to each other **Wang et al (2014)**. In this study 14\45 patients were having hypertension (31.1%) of all cases. Only 2 cases had pulp stone (14.2%), while 6 cases (42.8%), which is the higher percent showed characteristic vaculation. There was no statistically significant difference between mean stone areas in hypertensive and none hypertensive subjects. More investigations are needed to verify or deny the relation between hypertension and pulp vaculation. **Segura-Egea et al (2010)** disagreed with our findings who concluded that prevalence of endodontic treatment is not significantly

different in hypertensive patients compared with control subjects without hypertension.

No statistically significant difference of mean ionized calcium (Ca^{2+}), alkaline phosphatase (ALP), red blood cells (RBC), platelets (PLT), prothrombin time (PTT), (INR) and hemoglobin (HGB) levels between the two groups; this finding confirms that all persons in both groups are healthy, fulfilling the inclusion criteria and the patients do not suffer from any disease affecting blood.

Diabetics group showed statistically significant higher mean fasting blood glucose and HbA1c than control group. (Fasting Blood Glucose (mg/dl) mean ± SD is 96 ± 9.9 control/168.2 ± 41 diabetic P-value <0.001), (HbA1c (%) mean ± SD is 5.7 ± 0.3 control/ 8.6 ± 1.8 diabetic P-value <0.001) respectively. These results indicate that none of patients reached continuous controlled diabetes, which explain absence of normal pulp in group A. **Inagaki et al (2010)** found similar results in 30 week old rats that showed higher fasting blood glucose level than that of non diabetic rats.

Diabetic group showed statistically significantly higher mean WBC than control group. This coincides with **Vozarova et al (2002)** who found increased WBCs in type 2 diabetes. This might be because the presence of focal area of inflammation in the dental pulp induced by diabetes that stimulates production of further WBCs, as well as other areas that might be scattered overall the body.

Mann-Whiteny U test was made for the comparison between stone areas in females and males and showed that females had statistically significantly higher mean stone areas than males P-value 0.030 (Significant

at $P \leq 0.05$). This result agrees with a retrospective study made by **Sisman et al (2012)** in which they found that pulp stones were detected more in teeth examined in females (10.56%) than in males (4.44%) with significant difference between the genders ($p<0.05$). But **Nayak et al (2010)** announced opposite results in their radiographic research that aimed to find correlation between systemic disorders and pulp stones. They revealed that although the occurrence of pulp stone was higher in females than males. However, there was no statistically significant difference between sexes ($P>0.05$).

Results of Mann-Whiteny U test revealed that Non-smokers in group A showed statistically significantly higher mean stone areas than smokers. This is because the small no of smokers taken in the study in comparison to the non smoker. In contrast **Punit and Sheela (2013)** found 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. For that further researches are required.

As there was only one case of bruxism had stone in group A in the study it gives no statistically significant difference between mean stone areas in subjects with and without bruxism. Thoroughgoing studies are needed in this field to determine the relation between bruxism and pulpal stones.

In our study we measured alkaline phosphatase ALP concentration in blood which gave us relatively normal values of all control and diabetic samples (except 6 cases in group A had high values (>135U/L), these patients are representing 13.3% of diabetic group) but with no relation to histological findings in pulpal tissues. **Inagaki et al (2010)** studied the ALP

activity that was indicative factor for the relation between high glucose levels and hard tissue forming function of dental pulp cells. **Catanzaro et al (2006)** found at 30 days of diabetes a significantly increased dental pulp ALP. These results pointed that diabetes leads to local increase of the activity of ALP in dental pulp, but not affecting the blood concentration.

Ionized calcium test in blood was made for all patients. Ionized calcium is the active form and constitutes about (50%) of the serum calcium. In clinical view: It is only the ionic calcium which matters as they are active. Sixty percent (60%) of diabetic group range in a normal values (1.1-1.3 mmol/l). Forty percent (40%) of specimens were having low values (0.7-0.99 mmol/l). In our study there was no significant relation between ionized calcium values and degree of dental pulp calcification or stone formation. This may be due to the low calcium level found in the specimens, which opposes the results revealed by **Nakade et al (2001)** as they found that high extracellular calcium level regulates bone morphogenetic proteins (BMPs) and type I collagen synthesis in osteoblastic cells and significantly increases cell proliferation.

Spearman's correlation coefficient was done for the correlation between stone area and age. It revealed no statistically significant correlation between stone areas and age. That is due to patients selected in the study have nearly similar mean age that statistically does not make significant difference.

There was no statistically significant correlation between stone areas and diabetes duration P-value 0.499 (Significant at $P \leq 0.05$). This can be explained because the subjective data of the duration that were gained from

the patients, this increases the liability of inaccuracy as each patient gave the history from onset of discovery not affection. **Inagaki et al (2010)** had opposite result who found larger calcified particles in 30 week old rats than 5 week old rats.

In our study there was a statistically significant positive (direct) correlation between stone areas and HbA1c i.e. an increase in HbA1c was associated with an increase in stone areas. This coincides with results revealed by **Inagaki et al (2010)** as their data indicated that hyperglycemia affected the number and size of calcified particles in rat dental pulp.

There was a statistically significant negative (inverse) correlation between stone areas and HGB i.e. an increase in HGB is associated with a decrease in stone areas. Further investigations are needed in this field in order to understand the relation between hemoglobin concentration and total area of pulp stone in diabetic patients.

Summary and conclusion

The present study aimed to evaluate the effect of diabetes mellitus on dental pulp of permanent teeth, by recording the histopathological changes occurring in these teeth.

Sixty five recently extracted teeth were histopathologically evaluated, including forty five teeth extracted from patients having diabetes for at least (8-10) years (group A) and 20 teeth extracted from healthy patients (group B). All patients signed a consent sheet. Patients selected were of age ranging between (20-40) years old (mean age 30 years). Thorough clinical and oral examination was done for each patient with measuring of blood pressure. Habits like smoking and bruxism were recorded. Decalcification of extracted teeth was done and stained using a Hematoxylin and Eosin stain. Blood samples were taken from patients to be tested for Glycated hemoglobin HbA1c, Ionized Calcium level, Serum alkaline phosphatase ALP, Fasting blood sugar and complete blood picture. Histopathological examination of pulp chamber and root canals was done by light microscope to determine pulpal changes. Statistical analysis of numerical data was done.

All specimens of control group showed normal pulp. Group A revealed signs of inflammation, fibrosis, calcification and stones, partial necrosis and angiopathy. Group A showed statistically significant higher mean fasting blood glucose, HbA1c and WBC than group B. There was no statistically significant correlation between stone areas and age or diabetes duration. Females showed statistically significantly higher mean stone areas than males $P \leq 0.05$. There was a statistically significant positive (direct)

correlation between stone areas and HbA1c. There was a statistically significant negative (inverse) correlation between stone areas and HGB. There was no statistically significant correlation between stone areas and other laboratory investigations.

In conclusion, diabetes mellitus seems to be a causative factor of many modifications of dental pulp components that may be involved in histopathological changes. Calcification and stones are the most prevalent changes that can increase liability and need of teeth for endodontic treatment as well as jeopardize root canal therapy and increase the difficulties faced by the endodontist during the procedures of the treatment. It may eventually become possible to control these modifications at the early state of diabetes, and to follow new strategies for the success of pulp treatment.