JOURNAL OF DENTISTRY 43 (2015) 589–596

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/jden

Diabetes detrimental effects on enamel and dentine formation

M.A. Abbassy [a,b,c,d], I. Watari [b], A.S. Bakry [e,f,*], H. Hamba [g], Ali H. Hassan [a], J. Tagami [g], T. Ono [b]

[a] Department of Orthodontics, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia
[b] Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Sciences, Graduate School, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8549, Japan
[c] Global Center of Excellence Program, International Research Center For Molecular Science in Tooth and Bone Disease at Tokyo Medical and Dental University, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8549, Japan
[d] Alexandria University, Alexandria, Egypt
[e] Operative Dentistry Department, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia
[f] Conservative Dentistry Department, Faculty of Dentistry, Alexandria University, Egypt
[g] Cariology and Operative Dentistry Department, Graduate School of Medical and Dental Sciences, Tokyo Medical & Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8549, Tokyo, Japan

ARTICLE INFO

Article history:
Received 8 October 2014
Received in revised form
28 December 2014
Accepted 15 January 2015

Keywords:
Enamel thickness
Dentine thickness
Type 1 Diabetes Mellitus
Micro CT
Histomorphometry
Tooth mineral density

ABSTRACT

Objectives: Understanding morphological changes and mineral content of tooth hard tissues may influence dental treatment. In this study, the effect of Type 1 Diabetes Mellitus (T1DM) on tooth structure was examined.

Methods: Experimental T1DM was induced in 3-week old male Wistar rats ($n = 10$) by a single dose of 60 mg/kg body weight of Streprozotocin. All rats were injected with calcein twice during the experiment and sacrificed at the age of 7 weeks old. Micro-computed tomography (micro-CT) was used to determine the mineral density and thickness of enamel and dentine. Also, a histomorphometery study was conducted to detect the rates of dentine mineral apposition and formation. The examined area was in the crown analogue of the rat mandibular incisor parallel to the long axis of the mesial surface of the first molar. All results were compared using Students' *t*-test ($p < 0.05$).

Results: Results showed that the enamel and dentine thickness were significantly reduced (hypoplasia) and there was a significant reduction of the rate of dentine mineral apposition and formation, while there was no significant effect of the T1DM condition on the mineral density of enamel and dentine.

Conclusions: T1DM has a detrimental influence on the formation of enamel and dentine in the early growth stage.

Clinical significance: T1DM condition may alter treatment planning of orthodontic treatment as it is associated with decreased enamel and dentin thickness that may affect teeth size and their resistance to caries.

* *Corresponding author at:* Operative Dentistry Department, Faculty of Dentistry, King Abdulaziz University, P.O. Box 80209, Jeddah 21589, Saudia Arabia. Tel.: + 00966593944171; fax: + (00966) 012 6404166.
E-mail address: drahmedbakry@gmail.com (A.S. Bakry).
http://dx.doi.org/10.1016/j.jdent.2015.01.005
0300-5712/

1. Introduction

Type 1 Diabetes Mellitus (T1DM) is a chronic condition in which the pancreas produces little or no insulin necessary for glucose metabolism. It is one of the most prevalent systemic disorders affecting an increasing number of individuals globally. This disease exhibits various detrimental alterations on bones, and mineral metabolism.[1–3] However, there is scant information available on the possible effects exerted by the diabetic condition on tooth development and mineral content. Various clinical studies reported high caries prevalence in diabetic children when compared with healthy controls.[4] Previous research suggested that the aforementioned increased caries prevalence associated with type 1 diabetes may be due to alteration in the salivary gland functions that causes decreased salivary flow. Alternative speculations were that T1DM produced increased salivary glucose levels which may have increased permeability of the parotid gland basement membrane to the elevated blood glucose.[4]

Understanding the factors contributing to the increased caries susceptibility in young patients suffering from the diabetic condition, especially young orthodontic patients who have high probability for the development of caries during their orthodontic treatment may help dentists to plan suitable strategies for protecting such patients against the expected caries challenges. Moreover, it is of prime importance for dentists and orthodontists to explore any factors that might affect the dental tissues growth and thus the size of the teeth, which has a strong impact on the orthodontic treatment planning.[5]

In this study, non-destructive micro-computed tomography (micro-CT) was used to examine the influence of induced T1DM on enamel and dentine mineral density and thickness using an experimental rat model. Micro-CT uses a focused beam to provide higher resolution on small samples *in vitro*.[6] This method has been frequently used in experiments exploring bone[7,8] and is considered a promising technique for the assessment of tooth mineral density.[9–12] Moreover, a histomorphometric study was conducted to determine the effect of the T1DM condition on dentine formation and dentine mineral apposition rates in the continuously growing lower incisors of Wistar rats. This is an appropriate model for examining the effects of different factors on the development of hard tissues.[2] The tested null hypotheses in this study were that the T1DM condition will not adversely affect thickness, mineral density, and the rate of tissue formation and mineral apposition in enamel and dentine.

2. Materials and methods

2.1. Animals and experimental diabetic model

Animal protocols were approved by the Institutional Animal Care and Use Committee of King AbdulAziz University and Tokyo Medical and Dental University, and the experiment was carried out under the control of the University's Guidelines for Animal Experimentation.

Twenty 3-week old male Wistar rats were used for this study. They were randomly divided into two groups, the control group and the T1DM group; each group consisted of ten rats. The rats in the control group were injected intraperitoneally with a single dose of 0.1 M sodium citrate buffer (pH 4.5), while the rats in the T1DM group were injected intraperitoneally with a single dose of citrate buffer containing 60 mg/kg body weight of streptozotocin (STZ; Sigma Chemical Co., St. Louis, MO, USA).[2,13,14] All animals were fed standard rodent diet (Rodent Diet CE-2; Japan Clea Inc., Shizuoka, Japan) with free access to water. Body weights, the presence of glucose in urine and blood glucose levels were recorded on day 0, 2, 7, 14, 21 and 28 after STZ injection.

T1DM condition was diagnosed by the presence of glucose in urine and elevated blood glucose concentration. The urine was tested using reagent strips (Uriace Ga; TERUMO).[3] Blood samples of the rats were obtained *via* tail vein, and blood glucose levels were determined using a glucometer (Ascensia Brio. Bayer Medical). Positive urine test and a blood glucose level greater than 200 mg/dl was considered diagnostic for T1DM.[2]

2.2. Calcein administrations and sections preparation

All rats were subcutaneously injected with 50 mg/kg body weight calcein fluorescent marker on day 21 and day 28 after STZ injection. At the end of the study, all animals were anaesthetized and sacrificed by transcardiac perfusion by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The right mandibles were removed and fixed in the same solution. After being embedded in polystyrene resin (Rigolac, Nisshin EM Co. Ltd., Tokyo, Japan), undemineralized ground mesial sections were cut using water-cooled diamond saw microtome (1600 Microtome, Leitzwetzlar, Germany) parallel to the long axis of the rat molars just 2 mm to the mesial surface of the first lower molar crown; the distal second cut was done 2 mm distal to the crown of the first molar. The specimen mesial surface was then ground flat with water-cooled silicon carbide discs (600- and 1200-grade papers; Buehler) till it is possible to observe the two mesial canals and two mesial pulp chamber horns of the first molar. The ground mesial surface was glued on a glass slide and the same grinding procedures were repeated from the distal surface until we can observe the two mesial canals and two mesial pulp horns of the first molar from the distal side. The obtained specimen is then wet-polished using diamond paste (1 μm; Buehler) to obtain a highly polished surface.[15]

2.3. Analysis of histomorphometric indices

Dentine formation indices in control and T1DM groups were determined in the crown analogue area parallel to the long axis of the mesial surface of the first molar. A digitizing morphometry system was used to measure the dentine formation indices. The system consisted of a confocal laser scanning microscope (LSM510, Carl Zeiss Co. Ltd., Jena, Germany), and a morphometry program (LSM Image Browser, Carl Zeiss Co. Ltd., Germany). Dentine formation indices included dentine mineral apposition rate (μm/day) and dentine formation rate ($\mu m^3/\mu m^2$/day). This method was