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Complication of Type 1 Diabetes in Craniofacial and Dental Hard Tissue

Ippei Watari, Mona Aly Abbassy, Katarzyna Anna Podyma-Inoue and Takashi Ono

Additional information is available at the end of the chapter

Abstract

Diabetes mellitus (DM) is a chronic systemic disease arisen under the conditions when the body cannot produce enough insulin or cannot use it effectively. Type 1 diabetes is caused by an autoimmune reaction, where the body's defense system attacks the insulin-producing β-cells in the pancreas. Type 1 diabetes incidence has been rising all over the world, especially under the age of 15 years. There are strong premonitions of geographic difference; however, the overall annual increase in a number of affected population is estimated to be approximately 3%.

Under these circumstances, detailed understanding of the influence of type 1 diabetes on various organs is integrant. The systematic diseases have been seen to have a considerable effect on bone. As such, diabetes also exerts some degree of influence on bone in general. Hyperglycemia or impaired glucose metabolism has a number of detrimental effects on bone metabolism; for example, it is well documented that bone mass decreases and rate of bone fracture risk increases in type 1 diabetes patients. Nonetheless, there are few reports describing the influence of type 1 diabetes on hard tissue in craniofacial region.

From a dental clinical perspective, uncontrolled diabetic condition is thought to be one of the main causative factors of increased risk of inflammation and dental caries that lead to tooth loss and may also increase the risk of cardiovascular disease or preterm birth. However, there are only few reports focusing on type 1 diabetes complication in oral and maxillofacial region. Thus, in this chapter, we summarize the complication of type 1 diabetes in craniofacial hard tissue. Based on our previous data, type 1 diabetes lead to the retardant effects in cranium, mandible, and teeth during early growth period. This information is of critical importance not only for the better

understanding of the type 1 diabetes complication in jaw or teeth but also for the development of efficient treatment and prevention of oral diseases in type 1 diabetes patients.

1. Introduction

Type 1 diabetes is a chronic and a complex autoimmune disease arisen primarily due to β-cell destruction. Historically, type 1 diabetes was considered as a disorder in children and adolescents, but now it is known that symptomatic onset of type 1 diabetes may occur at any age. Three major symptoms, polydipsia, polyphagia, and polyuria along with overt hyperglycemia, are a diagnostic hallmark in young type 1 diabetes patients. Exogenous insulin replacement is needed immediately after the onset of type 1 diabetes and should be kept throughout their lifetime for survival.

To prevent the diabetic complication, patients with type 1 diabetes require a strict control of blood glucose level.

Although type 1 diabetes can be diagnosed at any age, it is one of the most common chronic diseases of childhood. Its prevalence increases between the ages 5 and 7 years or near puberty [1].

It has been reported that the incidence of type 1 diabetes is increasing worldwide for several decades [2] and it is likely to have been most pronounced in children aged 4 years and younger [3]. If these trends continue, the total prevalence of people with type 1 diabetes will increase in the coming years [4].

A continuous hyperglycemia in type 1 diabetes leads to various chronic complications. Recently, official healthcare providers have paid more attention to the prevention of disabling chronic complications, such as diabetic retinopathy, nephropathy, neuropathy, and atherosclerosis with cardiovascular disease, and much more attention has been paid for adverse bone metabolism in type 1 diabetes. [5] In this review, we provide a brief overview on the effects of type 1 diabetes on both bone in general and hard tissue in craniofacial region.

2. General effects of type 1 diabetes on bone

The relation between diabetes and bone metabolism has been considered for a long time; however, many questions still remain hidden and unclear. Pathophysiology of diabetes arises from the insufficient insulin action, and such insulin action may have an influence on the bone metabolism directly or indirectly. Clinically, it is well known that type 1 and type 2 diabetes are involved in an increased risk of fractures [6, 7]; on the other hand, bone mineral density

(BMD) is decreased in type 1 diabetes than in type 2 diabetes [7]. The reasons for this discrepancy are not fully understood. Indeed both type 1 and type 2 diabetes are the same in terms of an abnormal glucose tolerance, but pathological condition is different. In this part, we discuss diabetic osteopenia in type 1 diabetes from the viewpoints of insulin deficiency and hyperglycemia.

2.1. Insulin deficiency and bone metabolism

It is widely recognized that bone volume and bone quality are decreased in type 1 diabetes patients, and it is thought that insulin has a pivotal role in bone formation [7]. In animal experiment, streptozotocin (STZ)-induced type 1 diabetes rat or mouse showed a decrease in bone volume (BV) and bone fragility by the decrease of bone formation [8–10].

In insulin receptor substrate-1 (IRS-1)-deficient mouse, osteoblast differentiation and function were impaired, and as a result, there is a decrease in BV [11].

Remarkable hyperglycemia exists with insulin deficiency in the type 1 diabetes model animals, and it seems to be thought that not only the insulin deficiency but also the hyperglycemic condition gives some influences on bone metabolism. On the other hand, it appears that decrease in anabolic action at the osteoblasts level in type 1 diabetes is the main cause of the bone metabolism disorder by serial animal experiments in which the disorder of glucose metabolism is slight under the normal breeding condition in IRS-1- or IRS-2-deficient mice. On the basis of these findings, one should consider the rise in onset, osteoporosis, and bone fracture frequency of the osteoporosis in type 1 diabetes mellitus depends on an osteoplasty disorder by the insulin deficiency.

2.2. Bone Mineral Density (BMD) in type 1 diabetes

In 1948, Albright and Reifenstein described for the first time the association between diabetes and reduced bone mass [12]. In 1976, Levin et al. demonstrated that almost 50% of the patients with type 1 diabetes had a reduction of BMD at the wrist [13]. Since then, many papers have been published. BMD seems to be reduced in patients with type 1 diabetes in most [14–17], but not all [18, 19]. The studies concerning the bone metabolism in type 1 diabetes can be categorized into two groups: 1) studies evaluating bone metabolism in diabetic children and adolescents who did not reach the peak of bone mass yet and 2) studies evaluating bone metabolism in adults who developed type 1 diabetes after having reached peak of bone mass.

It seems to be difficult to study bone metabolism in such population as children/adolescents whose skeleton is still in the way of growing. Moreover, the majority of studies included the children/adolescents at different stage of puberty and, therefore, at different stages of acquisition of bone mass. This probably has been one of the main reasons for the lack of concordant results about the impact of diabetes on growing bones.

Some reports showed no differences in BMD between type 1 diabetic children/adolescents and their peer without diabetes [20–26]. However, in other studies, low bone mineral content (BMC) and low BMD both at spine and at femoral neck in type 1 diabetic children/adolescents

[27–33] have been described. Moreover, some longitudinal studies demonstrated a significant reduction of either lumber spine or femoral neck BMD in diabetic patients after 2–4 years of follow-up, despite normal BMD at baseline [20, 23]. Therefore, it seems that type 1 diabetes, appeared in childhood, may alter the acquisition of bone mass that can be registered in youth ages or later in adult life.

Indeed, the majority of studies, performed on the type 1 diabetes adults, consistently showed a reduction of BMD either at lumbar spine and/or at femur [34, 35, 36–40]. Only a few studies [41–43], which were conducted on small groups of diabetic patients (less than 40 cases), were discordant. Vestergaard et al. [44] having analyzed 80 studies regarding bone density in diabetes has proved in his meta-analysis that type 1 diabetes patients have lower BMD than the people without diabetes. Frequency of reduced BMD in type 1 diabetes varies largely from 3 to 40% [36–40]. Eller-Vainicher et al. [45] reported that about 30% of 175 type 1 diabetes patients had low bone mass (osteopenia/osteoporosis) at spine and/or femur, which was significantly higher in comparison with healthy controls.

2.3. Fracture risk in type 1 diabetes

In type 1 diabetes patients, the frequency of lifetime fractures at any site has been reported to be increased as compared to counterparts without diabetes. The meta-analysis of Vestergaard et al. [44] demonstrated a 6.94-fold increased risk of hip fracture in type 1 diabetes. Further, *Zhukouskaya et al.* [45] reported that type 1 diabetes patients were found to have an increased prevalence of asymptomatic vertebral fractures as well, which have been observed in 25% of diabetic subjects. In conclusion, there is strong evidence that bones in type 1 diabetes patients are characterized by poor mineralization and smaller and thinner size with reduced bone strength and quality, which can lead to a higher fracture incidence at any site, predominantly at femoral neck.

2.4. Association between hyperglycemia and bone metabolism in diabetes

Type 1 diabetes is caused by absolute lack of insulin, and insulin has anabolic effect on bone. However, not only insulin but also hyperglycemia has some influence on the bone metabolism. In in vivo study, it is difficult to evaluate the influence on bone metabolism by hyperglycemia or insulin deficiency separately, so the influence of hyperglycemia on bone is considered at a cell level mainly.

In an experiment of osteoblastic cell, it was reported that the differentiation and function of osteoblastic cell were suppressed under osmolality-adjusted hyperglycemic condition [46].

In our previous experiment using MC3T3-E1 cell line, osteoblastic cells were cultured in medium containing normal (5.6 mM) or high (10, 20, or 30 mM) glucose with or without bone morphogenic protein 2 (BMP-2). Runx2 mRNA expression, which is a key transcription factor associated with osteoblast differentiation, was affected by glucose concentration and culture duration independently of the absence or presence of BMP-2 in the culture. (Fig. 1) [47]. Moreover, we could find both GLP-1 receptor (GLP-1R) and GIP receptor (GIPR) m RNA expression in osteoblastic cell first time ever (Fig. 2), and mRNA expression level of GLP-1R

and GIPR were regulated by glucose concentrations in cells undergoing the differentiation induced by BMP-2 (Figs. 3, 4). GLP-1 or GIP belong to the incretin family. They both play important roles in regulating insulin secretion from pancreatic β-cells. GIPR and GLP-1R, the receptors of GIP and GLP-1, are expressed in various tissues, with a significant amount expressed in pancreas. Previous reports showed that GIPR is expressed in osteoblastic cells, but no study regarding GLP-1R expression had been conducted [48]. Although osteoblastic cells were thought to express a functional receptor for GLP-1, there is no direct evidence for the mRNA and protein expression of GLP-1R in these cells. GIP is known to have direct effects on bone, whereas the effects of GLP-1 on bone metabolism are mediated by thyroid hormone. [49] Our RT-PCR analysis revealed that MC3T3-E1 cells express GLP-1R and GIPR, suggesting that GLP-1 may directly affect bone, similar to GIP (Fig. 4). GLP-1R and GIPR are well-known G protein-coupled receptor (GPCR) and are potential targets for drug discovery [47]. It has been reported that the administration of insulin and thiazolidinediones increases fracture risk, whereas inhibitors of dipeptidyl peptidase-4 (DPP-4) were associated with reduced fracture risk. DPP-4 inactivates GLP-1, and its inhibitors improve glycemic control in patients with type 2 diabetes by preventing incretin degradation [50]. These findings show that GLP-1R links bone metabolism and glucose metabolism in osteoblasts and that GLP-1 might be a potential therapeutic target in bone diseases.
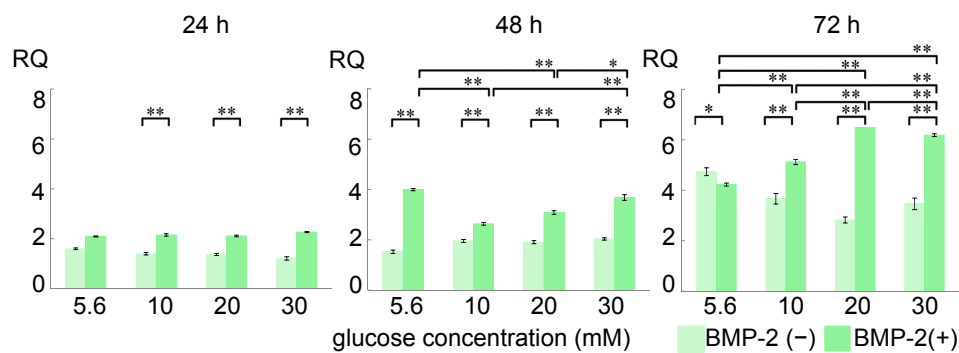

Figure 1. Effects of the glucose concentration on Runx2 mRNA expression. MC3T3-E1 cells were cultured in medium containing 5.6 (normal), or 10, 20, and 30 mM (high) concentrations of glucose in the absence or presence of bone morphogenetic protein-2 (BMP-2). Runx2 mRNA expression was determined after 24, 48, and 72 h of culture. Values are the means ± standard error of the mean (SEM) (n = 4/group). *P < 0.05 and **P < 0.01.
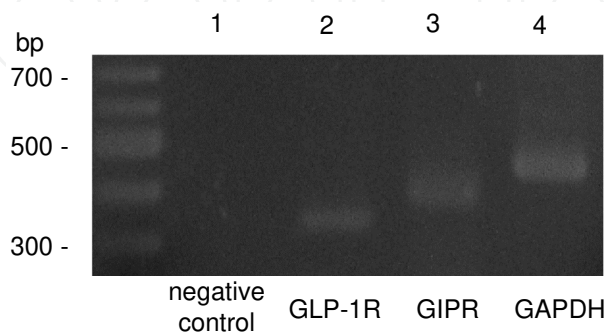

Figure 2. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of glucagon-like peptide-1 receptor (GLP-1R) and glucose-dependent insulinotropic polypeptide receptor (GIPR) mRNA expression in MC3T3-E1 cells. Lane 1, negative control; lane 2, GLP-1R (337 bp); lane 3, GIPR (382 bp); lane 4, GAPDH (452 bp).
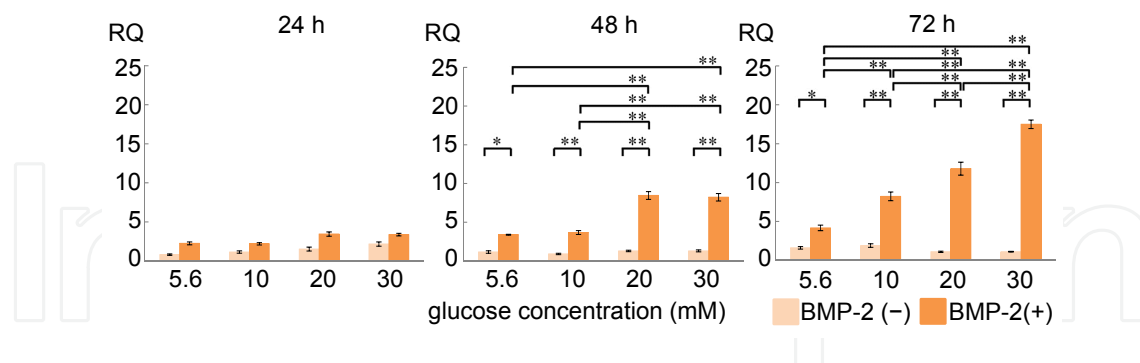
Figure 3. Effects of the glucose concentration on glucagon-like peptide-1 receptor (GLP-1R) mRNA expression. MC3T3-E1 cells were cultured in medium containing 5.6 (normal), or 10, 20 and 30 mM (high) concentrations of glucose in the absence or presence of bone morphogenetic protein-2 (BMP-2). GLP-1R mRNA expression after 24, 48, and 72 h of culture. Values are the means ± standard error of the mean (SEM) (n = 4/group). *P < 0.01.
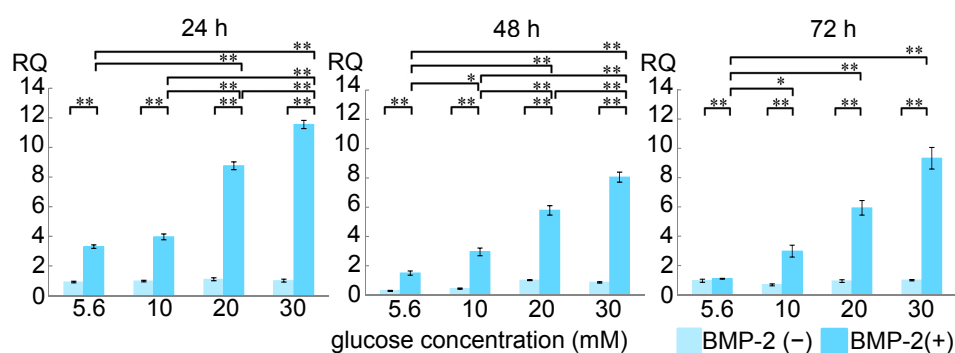

Figure 4. Effects of glucose concentration on glucose- dependent insulinotropic polypeptide receptor (GIPR) mRNA expression. MC3T3-E1 cells were cultured in medium containing 5.6 (normal), or 10, 20, and 30 mM (high) concentrations of glucose in the absence or presence of bone morphogenetic protein-2 (BMP-2). GIPR mRNA expression after 24, 48, and 72 h of culture. Values are the means ± standard error of the mean (SEM) (n = 4/group). *P < 0.01.

3. Effect of type 1 diabetes on craniofacial complex

Diabetes is one of the systemic diseases affecting a considerable number of patients worldwide [51]. Numerous clinical and experimental studies on the complications of diabetes have demonstrated extensive alterations in bone and mineral metabolism, linear growth, and body composition [52]. As we mentioned in the previous section, depletion of insulin in type 1 diabetes causes a reduction of bone composition, delay in fracture healing, and reduction of BMD in general. A long list of literature was dedicated to study the influence or complications of type 1 diabetes on general bones. However, there are few reports discussing the effects of type 1 diabetes on the craniofacial complex which is regulated by hormones, nutrients, mechanical forces, and various peripheral growth factors.

In craniofacial region, it is well known that bone metabolism in growth period is really intricate because there are mosaic growth sites where bones grow at different rates or mature at different times, which also depend on each individual's growth stage, and the response to growth disruption is much more complicated than that of the appendicular skeleton. There are a few studies diabetes may significantly affect the bone remodeling process which is observed during treatments involving the application of mechanical or functional force to the craniofacial complex and the teeth as those applied during orthodontic tooth movement. Moreover, it is likely that the type 1 diabetes may have altered the growth of patients due to insulin deficiency and consequently led to skeletal mutation is it mutation or maturation?

Type 1 diabetes is well recognized in the endocrine disorders,, and a peak of onset is concentrated in childhood and adolescence, characterized by hyperglycemia as a cardinal biochemical feature that leads to several impairment of physical and emotional developments. There are some reports focusing on the altered bone remodeling in type 1 diabetes, which indicates the reduction of osteoblast activity or function. Bone mass decrease and rate of bone fracture risk increase have been often seen in type 1 diabetes patients. Impaired glucose metabolism results in adverse effects on bone metabolism, especially in type 1 diabetes patients who suffer from decreased bone mineral density (BMD) and increased risk of fractures. The pathophysiological mechanisms of increased risk of fracture in diabetes patients are divided into two reasons: osteopenia caused by decreased BMD and increased risk of fall and traumas caused by peripheral diabetic neuropathy. However, there are few reports about hard tissue in craniofacial region, in other words, cranium, maxilla, mandible, and teeth.

The aim of this chapter is to discuss the complexity of the dento-alveolar system and how it was affected by type 1 diabetes.

3.1. Effect of type 1 diabetes on bone in craniofacial region

There are two processes of bone formation: "intramembranous ossification" and "endochondral ossification." Endochondral ossification is a cartilage bone formation and it occurs in a replacement process within the cartilage models of the embryo and infant. Intramembranous bone forms through the activation of the osteoblastic cell or specialized bone forming cell in one of the layers of the fetal-connective tissue. The bones of the cranial vault, the face, and the clavicle are formed by the style of intramembranous ossification. All the other bones are formed in the manner of cartilage ossification. The bones formed by intramembranous ossification are the mandible, the maxilla, the premaxilla, the frontal bone, the palatine bone, the squamous part of temporal bone, the zygomatic bone, the medial plate of the pterygoid process, the vomer, the tympanic part of the temporal bone, the nasal bone, the lacrimal bone, and the parietal bone. The original pattern of intramembranous bone changes with progressive maturative growth when these bones begin to adapt to environmental influences. This accounts for deformities due to malfunction, disease, and other environmental factor [53].

3.2. Causes of general growth problems

It is thought that growth disturbance can be associated with specific anatomic or functional defects. Some kinds of endocrinal or metabolic disorders are known to cause a systemic growth disorder. Also, genetic, nutritional, or environmental factor can be the causes of growth disturbance. Disturbances in somatic growth show themselves in retardation or acceleration of the skeletal system, including the facial and cranial bones. Causes of growth problems usually fall into the following categories [54]:

- familial short stature;

- constitutional growth delay with delayed adolescence or delayed maturation;

- illness that affects the whole body (systemic disease);

- endocrine disease (hormonal disorder); and

- congenital problems in the tissues where growth occurs.

3.3. Effect of type 1 diabetes on bone and growth

Concerning juvenile diabetes, previous report about hand-wrist radiographs [55]. showed that usually, there is a delay in the development of appearance or ossification center of the carpal bone. These defects seem to occur twice as frequently in boys than in girls, and the total incidence of juvenile diabetes patients with abnormalities and developmental disorders was 24.3%.There was also a delay in the growth of bone, in 51% of diabetic males and in 60% of diabetic females. The trend of growth retardation in bone was large. The longer the disease duration of diabetes, the shorter the bone growth will be. Bone mass reduction in diabetic patients has been explained by the decrease in the proliferative capacity of fibroblasts. In addition, premature aging of all cells has been suggested as the basis for diabetes problems, which is believed to lead to early osteopenia. The yearly bone loss was reported to be 1.35% in patients with type 1 diabetes [56]. Moreover, reduction rate of bone mineral, along with the condition worsened in diabetes, was significantly faster despite of an increase in insulin dosage, when compared with patients with unchanged or improved insulin secretion. It was considered that exogenous insulin administration cannot fully compensate for the decrease in the endogenous insulin secretion. In addition, according to these studies, the bone resorption in patients with type 1 diabetes were increased, and vitamin D_3 deficiency associated with the disease were not observed. Vertebral bone density has been studied in type 1 diabetic children [56]. In diabetic children, it has been found that the cortical bone density decreases slightly but significantly compared with control. The decrease in the cortical bone mineral density in diabetes did not correlate with age, gender, the duration of the diabetes, or glycosylated hemoglobin concentration. These results suggested that in children with uncomplicated type 1 diabetes, decreased vertebral bone density is a minor abnormality that affects only cortical bone [55].

3.4. Outline of studying the effect of type 1 diabetes on craniofacial growth

To examine the dynamic bone metabolism and structure of craniofacial bone in diabetes, it is critically important in understanding the growth aspect and bone metabolism of the mandible. The next parts of this chapter are trying to focus on the following points:

1. The effects of juvenile diabetes on general craniofacial growth and skeletal maturation.

2. Analysis of the pattern of association between craniofacial morphology and skeletal maturation.

3. Determination of the mineral apposition rate and the bone formation rate in diabetic rat mandible using histomorphometric analysis.

4. Analysis of the diabetic effects on tooth (enamel and dentin formation).

3.5. Experimental rat model for type 1 diabetes

It is well known that the streptozotocin-induced diabetic rat and the spontaneously diabetic BioBreeding rat were used as experimental type 1 diabetic models [57]. Pathogenesis of altered bone formation in long bones after inducing type 1 diabetes with streptozotocin (STZ) has been well documented [58, 59]. Streptozotocin-induced diabetes mellitus (STZ-DM) caused by the destruction of pancreatic β-cells and is similar to type 1 diabetes in human. It is characterized by mild-to-moderate hyperglycemia, glucosuria, polyphagia, hypoinsulinemia, hyperlipidemia, and weight loss. STZ-DM also exhibits many of the complications observed in human DM including enhanced susceptibility to infection and cardiovascular disease, retinopathy, alterations in angiogenesis, delayed wound healing, diminished growth factor expression, and reduced bone formation. [60].

3.6. Induction of type 1 diabetic condition in animal experiment

We studied various changes on craniofacial hard tissue under DM condition using streptozotocin (STZ)-induced DM rat model. Three-week-old male Wistar rats (n = 12) were used for this study. They were randomly divided into two groups, the control group and the diabetes group (DM group), and each group consists of six rats. The rats in the control group were injected intraperitoneally with a single dose of 0.1M sodium citrate buffer (pH 4.5), while the rats in the DM group were injected intraperitoneally with a single dose of citrate buffer containing 60 mg/kg body weight of STZ (Sigma Chemical Co., St. Louis, MO, USA) [58, 61–63]. All animals were fed on 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 days 0, 2, 7, 14, 21, and 28 after STZ injection. Diabetes condition was determined by the presence of glucose in urine and blood. The urine of the rats was tested using reagent strips (Uriace Ga; TERUMO) [64, 65]. Blood samples of the rats were obtained via vein puncture of a tail vein, and blood glucose levels were determined using a glucometer (Ascensia Brio; Bayer Medical). Rats with a positive urine test and a blood glucose level greater than 200 mg/dl were considered as diabetic. Time course of the animal experiment is shown in Fig. 5.
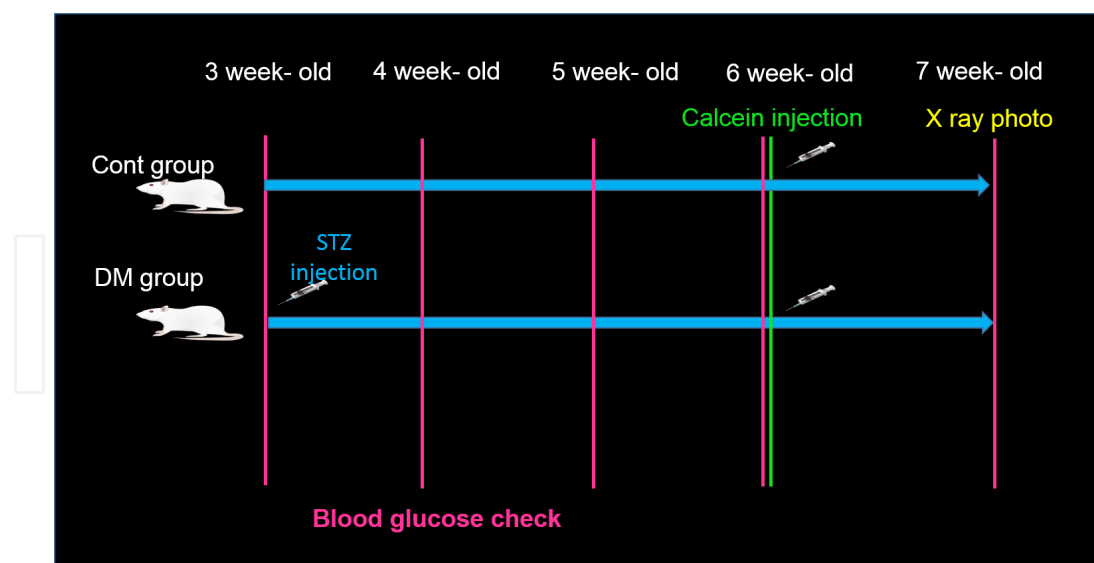
Figure 5. The time schedule of experiment

3.7. Evaluating the effect of type 1 diabetes on craniofacial growth in rat

Cephalometric analysis

Cephalometric measurements are still one of the most widely spread diagnostic aids crucial for the diagnosis of various abnormalities in the craniofacial complex [66].

The protocol for examining the cephalometric measurements in Type 1 diabetic rats involved the following steps:

1. Prior to each radiographic session, the rats were anesthetized with diethyl ether and intraperitoneally injected with 8% chloral hydrate using 0.5 ml/100 g of body weight.

2. After anesthesia, the rats were placed in the same way using specially designed apparatus to maintain standardized head posture and contact with the film (SGP-3; Mitsutoyo, Tokyo, Japan) where the head of each rat was fixed firmly with a pair of ear rods oriented vertically to the sagittal plane, and the incisors were fixed into a plastic ring. The settings of lateral and dorsoventral cephalometric radiographs were 50/55 kVp, 15/10 mA, and 20/60-sec impulses, respectively [68].

3. Then, a 10-mm steel calibration rod was incorporated into the clear acrylic table on which the animals were positioned for the radiographs.

All the radiographs were developed and scanned at high resolution by the same operator (Fig. 6). The cephalometric landmarks were derived from previous studies on rodents [68–70]. The selected linear measurements were then obtained (Table 1). To ensure reliability and reproducibility of each measurement, each distance was digitized twice and the two values were averaged. In our studies, evaluation of the craniofacial growth of diabetic rats at the age of 7 weeks was carried out using lateral and dorsoventral cephalometric radiographs. All of the data in each experiment were confirmed for the normal distribution; that is, Student's t-test

was used to compare the mean of each data recorded in the control group and in the DM group. All statistical analyses were performed at a 5% significance level using statistic software (v. 10; SPSS, Chicago, IL, USA).

Neurocranium	Mandible
Po–N: total skull length	Go–Mn: posterior corpus length
Po–E: cranial vault length	Ml–Il: anterior corpus length
Ba–E: total cranial base length	Co–Il: total mandibular length
So–E: anterior cranial base length	Co–Gn: ramus height
Ba–CB1: occipital bone length	**Transverse X-ray**
CB1′–CB2: sphenoid bone length	Go1–Go2: bigonial width
Ba–So: posterior cranial base length	C1–C2: maximum cranial width
Po–Ba: posterior neurocranium height	P1–P2: palatal width
Viscerocranium	Z1–Z2: bizygomatic width
E–N: nasal length	
Mu2–Iu: palate length	
CB2–Iu: midface length	
E–Mu1: viscerocranial height	

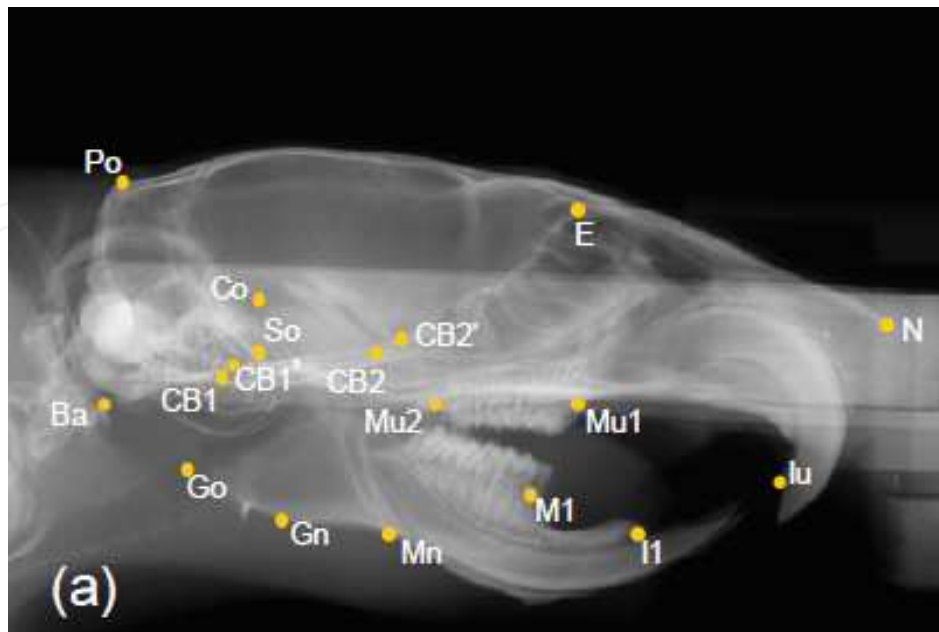**Table 1.** Measurements of craniofacial skeleton

Figure 6. Location of lateral cephalometric points on radiographs: (a) sagittal

3.7.1. Changes in the total skull

The size of total skull, denoted by Po-N, was found to be significantly smaller in the DM group than in the control group (Fig. 7).
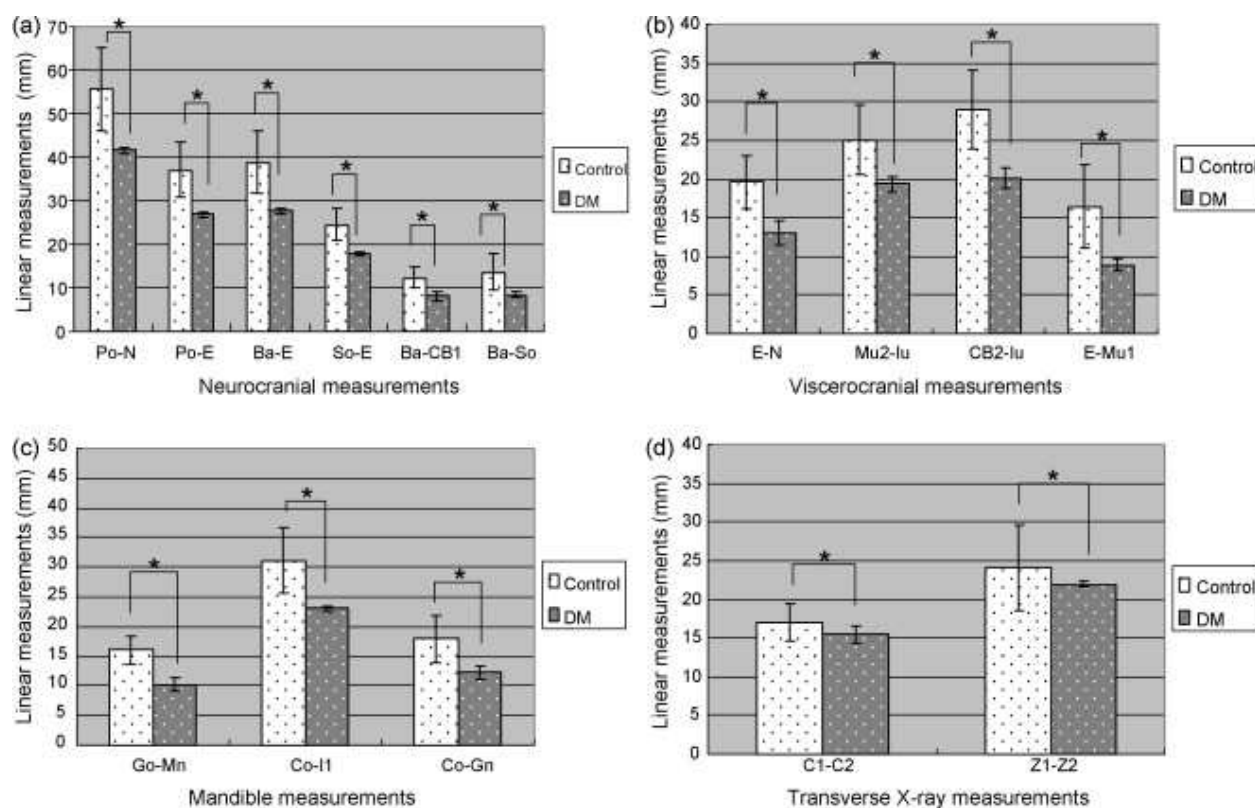

Figure 7. (A) Changes in the neurocranial measurements of the control and type 1 diabetes (DM) group. All the significant measurements are shown in this figure. Values are mean ± S.D. Significant differences between the two groups are marked with asterisks ($P < 0.05$). (B) Changes in the viscerocranial measurements of the control and DM groups. All the viscerocranial measurements are significant. Values are mean ± S.D. Significant differences between the two groups are marked with asterisks ($P < 0.05$). (C) Changes in the mandible measurements of the control and DM groups. Values are mean ± S.D. Significant differences between the two groups are marked with asterisks ($P < 0.05$). (D) Changes in the transverse X-ray measurements of the control and DM groups. Two measurements in the transverse X-ray were significant. Values are mean ± S.D. Significant differences between the two groups are marked with asterisks ($P < 0.05$).

3.7.2. Changes observed in the Neurocranium

Cranial vault length (Po-E), total cranial base length (Ba-E), anterior cranial base length (SoE), occipital bone length (Ba-CB1), and posterior cranial base length (Ba-So) were significantly shorter in DM group (Fig. 7), while the other dimensions showed no significant differences.

3.7.3. Changes in the Viscerocranium

All measurements of the viscerocranium, including the nasal length (E-N), palatal length (Mu2-Iu), midface length (CB2-Iu), and viscerocranial height (E-Mu1), showed a statistically significant decrease in DM group (Fig. 7).

3.7.4. Changes in the Mandible

In the DM group, the posterior corpus length (Go-Mn), total mandibular length (Co-Il), and the ramus height (Co-Gn) were significantly shorter than in the control group (Fig. 7); on the other hand, there were no statistical differences in the remaining dimensions.

3.8. Histomorphometric analysis of mandible

3.8.1. Fluorescent dyes used for double labeling in histomorphometric analysis

Fluorochromes are calcium-binding substances that are preferentially taken up at the site of active mineralization of bone known as the calcification front, thus labeling sites of new bone formation. They are detected using fluorescent microscopy on undecalcified sections. Labeling bones with fluorochrome markers provides a means to study the dynamics of bone formation. The rate and extent of bone deposition and resorption can be determined using double- and triple-fluorochrome labeling sequences. The sequential use of fluorochromes of clearly contrasting colors permits a more detailed record of events relating to calcification. Fluorochromes commonly used in mammals include tetracycline, calcein green, xylenol orange, alizarin red, and hematoporphyrin. Calcein gives bright green fluorescence when combined with calcium [71].

3.8.2. Calcein administrations and sections preparation

The detection of the double labeling involves the following steps:

- Rats are subcutaneously injected with 50 mg/kg body weight calcein fluorescent marker on day 21 and day 28 after STZ injection [72]. The time difference between the two injections was one week to be able to compare the amount of bone formed during this period (Fig. 8).

- All animals were sacrificed by transcardiac perfusion under deep anesthesia using 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4).

- Mandibles were dissected and fixed in the same solution for 24 h and embedded in poly-styrene resin (Rigolac; Nisshin EM Co. Ltd., Tokyo, Japan).

- Undermineralized ground frontal sections were processed to show the crown and both apices of buccal and lingual roots of the lower second molar [72].

3.8.3. Method of analysis of "Mineral Appositional Rate" and "Bone Formation Rate"

The bone around the lower second molar is centrally located within the mandibular arch, and the parallel alignment of the buccal and lingual roots is used as a precise reference when frontal sections are produced [73]. To conduct the histomorphometric analysis, it is essential to use a digitizing morphometry system to measure bone formation indices. The system consists of a confocal laser scanning microscope (LSM510; Carl Zeiss Co. Ltd., Jena, Germany) and a morphometry program (LSM Image Browser; Carl Zeiss Co. Ltd., Jena, Germany). Bone formation indices of the periosteal surfaces of the alveolar/jaw bone include mineral apposition
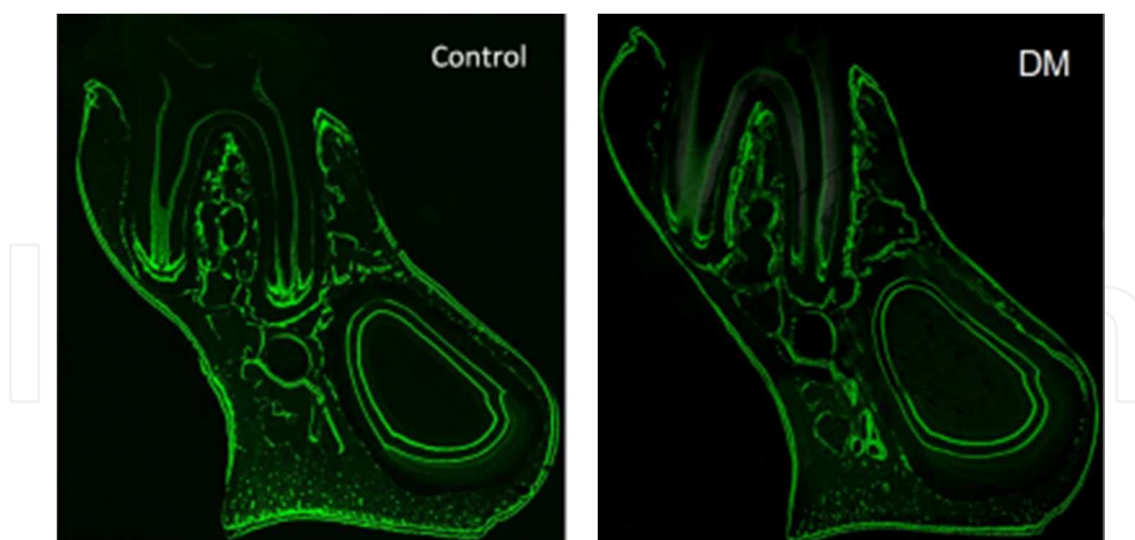
Figure 8. Frontal sections of the rat's mandibular second molar area. Control, control rat; DM, type 1 diabetes rat. Fluorescent labeling on the periosteal surface indicates new bone formation.

rate (μm/day) and bone formation rate (μm^3/μm^2/day), according to the standard nomenclature described by Parfitt and colleagues [74]. The calcein-labeled surface (CLS, in mm) is calculated as the sum of the length of double labels plus one half of the length of single labels (sL) along the entire endosteal or periosteal bone surfaces; that is, CLS = dL + 0.5sL [75]. The mineral apposition rate (MAR, in μm/day) is determined by dividing the mean of the width of the double labels by the interlabel time (7 days). The bone formation rate (BFR) is calculated by multiplying MAR by CLS [76]. Based on the reference line along the long axis of the buccal root, the area superior to the root apex was considered as an alveolar bone, while the area inferior to the root apex was considered as the jaw bone. The lingual side of the bone was excluded, because the existence of the incisor root might influence bone formation. The periosteal surfaces of the mandible were divided into four regions for analysis (Fig. 9).
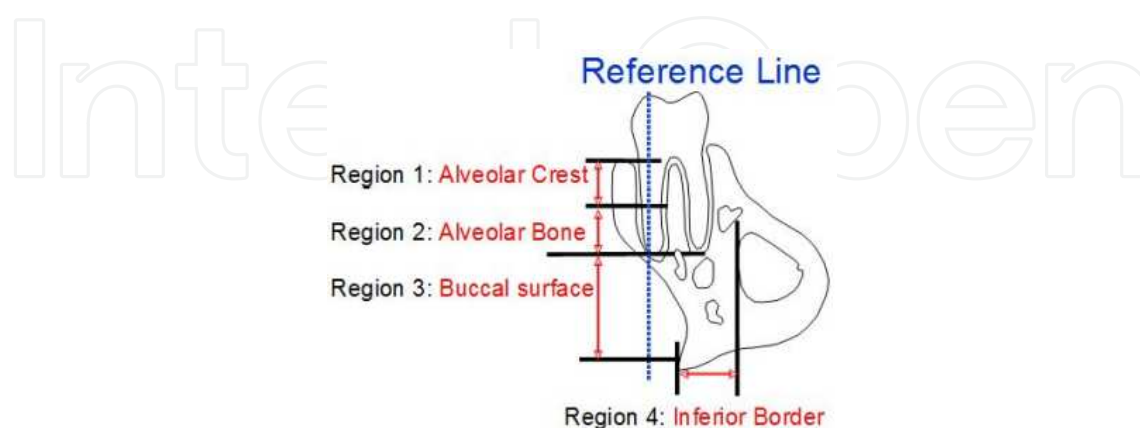

Figure 9. Schematic drawing of observation regions for dynamic bone histomorphometry. The periosteal surfaces were delimited into four areas: alveolar crest (region 1), alveolar bone (region 2), buccal surface of the jaw bone (region 3), and inferior border of the jaw bone (region 4).

3.8.4. Hitromorphometric indices

The obtained results in our study showed that in the alveolar bone (region 2), there was a significant decrease in the MAR (Fig. 10A) and the BFR (Fig. 10B) recorded in the DM group compared to the control group. However, in the alveolar crest (region 1), the MAR and the BFR in the control and the DM groups were not significantly different ($P < 0.05$). In the buccal surface (region 3) and inferior borders (region 4) of the jaw bone, the MAR (Fig. 10A) and BFR (Fig. 10B) were significantly suppressed compared with those in the control group ($P < 0.05$). Most of the periosteal surfaces in the mandibular regions of the control group showed significantly higher values recorded for the mineral apposition rate and the bone formation rate when compared to the DM group. These results agree with the previous studies that recorded diminished lamellar bone formation in DM rats' femur and may suggest an association between the DM condition and the decreased number and function of osteoblasts [61]. The alveolar crest region was the only region that did not show a significant difference in the MAR and the BFR parameters between the two groups; this may be attributed to the unique nature of this region exhibiting a highly intensive bone remodeling process especially during the teeth eruption that decreases toward the base of the socket [77]; however, further studies are needed to elaborate the detailed pattern of bone growth at the alveolar crest region.

3.9. Evaluating the type 1 diabetic effects on tooth

Type 1 diabetes exhibits various detrimental alterations on bones, and mineral metabolism [52, 58, 75]. 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 [78]. Previous studies suggested that the aforementioned increase in caries prevalence associated with type 1 diabetes may be due to alteration in the salivary gland functions resulting in decreased salivary flow. Alternative speculations were that type 1 diabetes produced increased salivary glucose levels which may have increased permeability of the parotid gland basement membrane to the elevated blood glucose. Understanding the factors contributing to the increased caries susceptibility of 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. Our study has employed the non-destructive micro-computed tomography (micro-CT) to examine the influence of induced type 1 diabetes 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. This method has been frequently used in experiments exploring bone and is considered as a promising technique for the assessment of tooth mineral density. In addition, a histomorphometric study was conducted to determine the effect of the type 1 diabetes 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
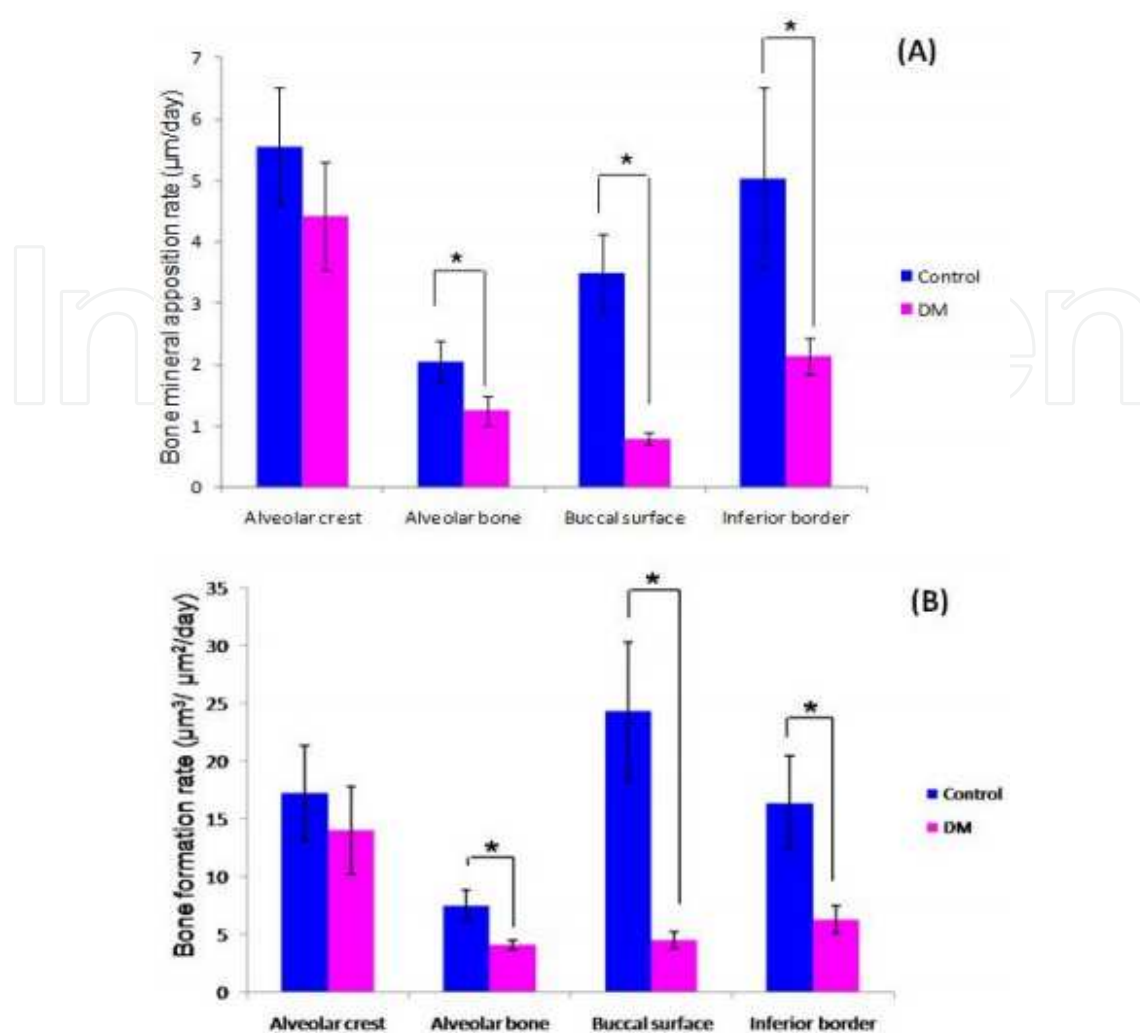
Figure 10. (A) Changes in the mineral apposition rate (MAR) of the mandible between the control group (red columns) and the type 1 diabetes mellitus (DM) group (blue columns). Alveolar crest (region 1, upper half of the tooth root, near the tooth crown). Alveolar bone (region 2, lower half of the tooth root, near the root apex). Buccal surface of the jaw bone (region 3). Inferior border of the jaw bone (region 4). The data are expressed as means ± S.D.; $n = 5$ for each group. Significantly different from controls, with *$P < 0.05$. (B) Changes in the bone formation rate (BFR/BS) of the mandible between the control group and the DM group. Alveolar crest (region 1, upper half of the tooth root, near the tooth crown). Alveolar bone (region 2, lower half of the tooth root, near the root apex). Buccal surface of the jaw bone (region 3). Inferior border of the jaw bone (region 4). The data are expressed as means ± S.D.; $n = 5$ for each group. Significantly different from controls, with *$P < 0.05$. In the buccal surface (region 3) and inferior borders (region 4) of the jaw bone, the MAR (Fig. 4A) and the BFR (Fig. 4B) are significantly suppressed compared with those in the control group ($P < 0.05$).

effects of different factors on the development of hard tissues. The tested null hypotheses in this study were that the type 1 diabetes condition will not adversely affect thickness, mineral density, and the rate of tissue formation and mineral apposition in enamel and dentine.

3.9.1. Calcein administration and section preparation for tooth observation

Rats were subcutaneously injected with calcein fluorescent marker (50 mg/kg body weight) on day 21 and day 28 after STZ injection. All animals were anesthetized 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; Leitz Wetzlar, 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) until it was 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 mm; Buehler) to obtain a highly polished surface.

3.9.2. Analysis of histomorphometric indices of tooth

Dentine formation indices in control and type 1 diabetes 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 (mm/day) and dentine formation rate ($\mu m^3/\mu m^2$/day). The method for the calculation of bone indices was modified from a method described by Parfitt et al. [74] The calcein-labeled dentine surface (CLS, in mm) was calculated as the sum of the length of double labels (dL) plus one half of the length of single labels (sL) along the entire dentine surface; that is, $CLS = dL + 0.5sL$ [17]. The mineral apposition rate (MAR, in μm/day and in μm^2/day) was determined by dividing the mean of the width of the double labels by the interlabel time (7 days). The dentine formation rate (DFR) was calculated by multiplying MAR by CLS [18]. For the measurements of mineral apposition rate, the average of 3 inter-label widths at a 100-μm interval was calculated for each sample.

Green fluorescent lines labeled with calcein fluorescent marker at two different time points showed that dentine formation took place between day 21 and day 28 in the control and type 1 diabetes groups (Fig. 11A and B). In the type 1 diabetes group, there were significant decreases in both mineral apposition and dentine formation rates (Fig. 11C and D) when compared to control group ($P < 0.05$).

Furthermore, our micro-CT results (details of method not shown) revealed that there was no significant difference in the enamel and dentine mineral densities between the control and experimental diabetes groups (Fig. 12). However, the type 1 diabetes group showed a significant decrease in the thickness of enamel and dentine surfaces when compared to the control group (Fig. 13) [79].
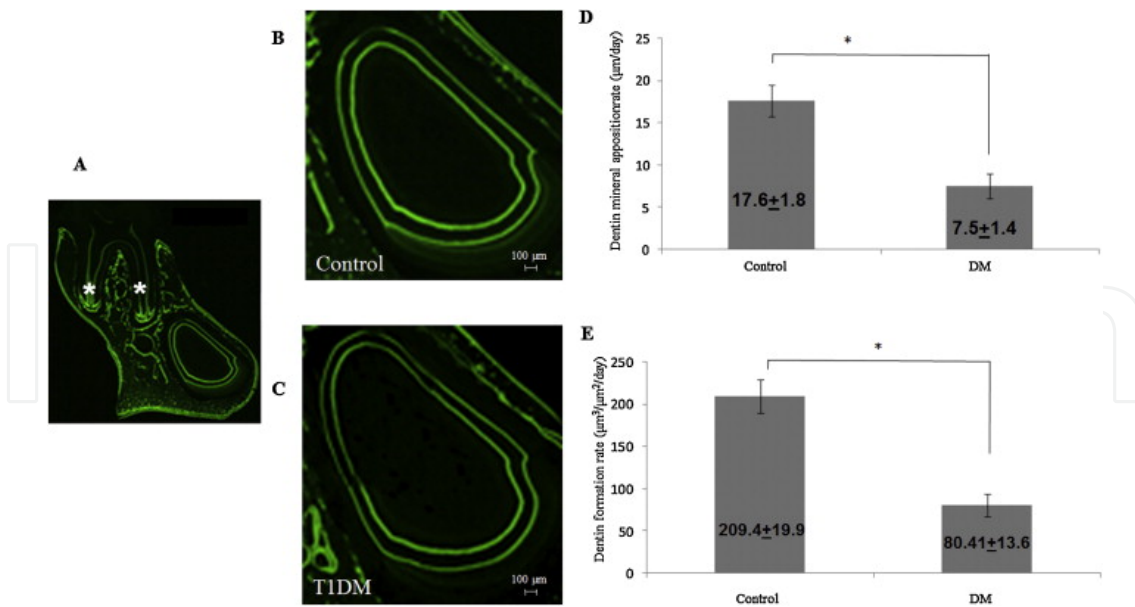
Figure 11. (A) Frontal section of the lower right mandible. *The lower first molar two roots that were considered the landmark for cutting all samples. (B, C) Frontal sections of the rat incisor mandibular first molar area. (B) Control; (C) T1DM. Fluorescent labelling indicates the new dentine formation. (D) The mineral apposition rate (MAR) of the dentine mandibular incisor for the control group and the T1DM group. The data are expressed as means ± SD. $n = 10$ for each group. Significant difference from controls, with *$P < 0.05$. (E) The dentine formation rate (DFR) of the dentine mandibular incisor for the control group and the DM group. The data are expressed as means ± S.D. $n = 10$ for each group. Significant difference from controls, with *$P < 0.05$).
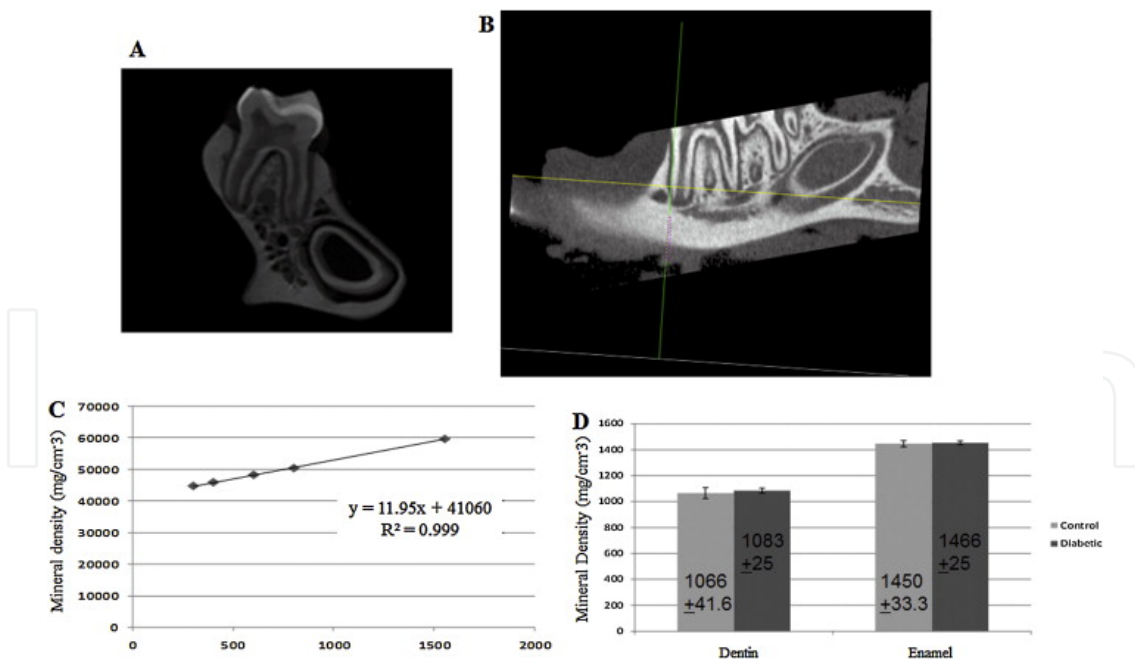
Figure 12. (A) Representative 3D reconstruction of the left mandible imaged by micro-CT. (B) The left mandible with the vertical reference line extending parallel to the mesial surface of the first molar. (C) Mineral density calibration curve based on the gray scale values obtained from the mineral reference phantoms (linear regression, R2 > 0.99). (D) Graph showing that there is no significant difference in the incisor enamel and dentine mineral densities between the control and T1DM groups.

Figure 13. (A) The micro-CT oriented image of the rat mandibular incisor showing the three zones (E1–E3) selected for e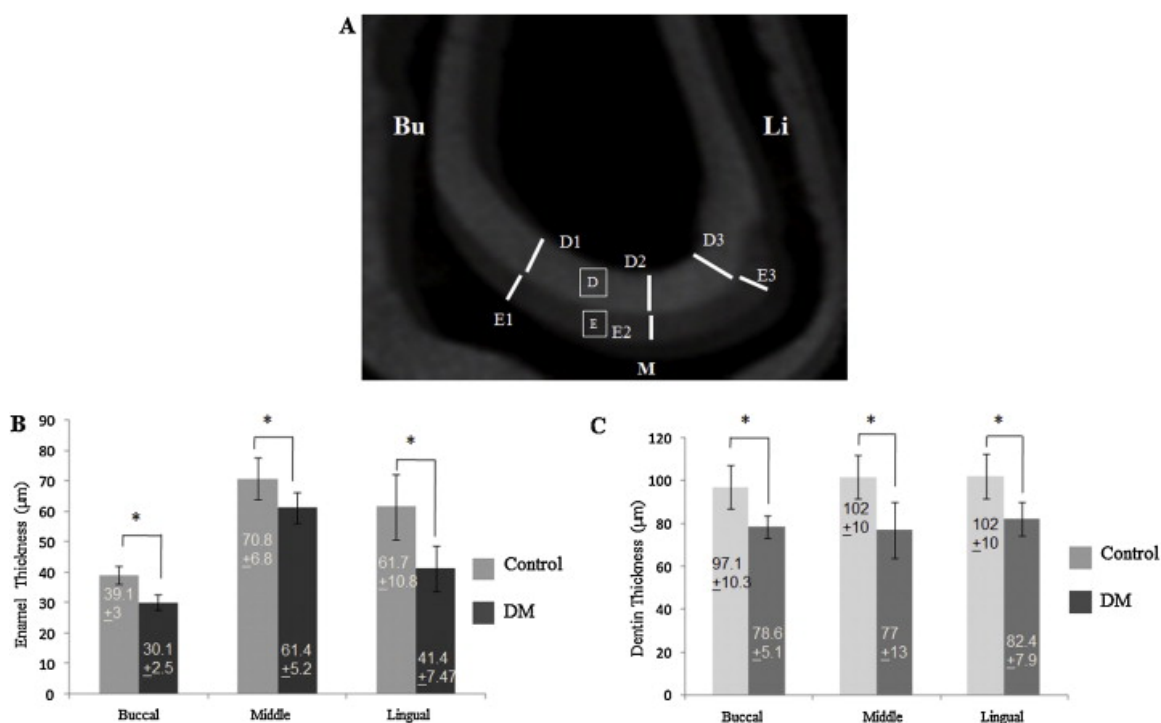valuation of enamel thickness and the three zones (D1–D3) selected for evaluation of dentine thickness. B-buccal; M-middle; Li-lingual. (B) The T1DM group shows a significant decrease in the thickness of enamel surface when compared to control group in the three different zones. (C) The T1DM group shows a significant decrease in the thickness of dentine surface when compared to control group in the three different zones ($P < 0.05$).

3.10. Suggested mechanism for the effect of diabetic condition on craniofacial complex

Growth of the craniofacial or maxillofacial complex is regulated by genetic and environmental factors [57]. For normal growth and morphogenesis of the cranial and maxillofacial complex, a proper regulation by hormones, nutrients, mechanical forces, and various general and local growth factor is essential. Type 1 diabetes causes a deteriorating growth and metabolic disorder of bone in both humans and experimental animals [58]. Since studies in humans are generally limited by small sample size, cross-sectional designs, uncontrolled variables, and often retrospective natures; it often performed more rigorous analyses using animal models [56]. We have observed the growth of the rat from 3 weeks of age to 7 weeks of age in our study. According to the previous craniofacial growth studies, this period corresponds to the initial stage of growth in humans [80, 81]. Consequently, STZ-induced DM models in our study were used to investigate the effects of type 1 diabetes on the development of craniofacial complex. These STZ-induced DM rats showed a significant reduction in the growth of a large portion of the unit of craniofacial hard tissues compared with control rats, but regarding the rest of the craniofacial skeletal units (sphenoid bone length, posterior neurocranium height, anterior corpus length, bigonial width, and palatal width), no significant difference were observed between the control and the STZ-induced DM groups. In general, craniofacial skeletal growth was significantly lower in STZ-induced DM group compared to controls in all three dimensions. The previous study investigated the DM effect exclusively on the growth of

the mandible and suggested that the diabetic condition had a differential effect on the osseous components and/or its associated non-skeletal tissues. They discussed that disharmony of the mandibular growth was due to the condition of the DM, such as renal failure, anemia, body weight change, or alteration in the food-intake qualities [58]. Thus, we hypothesize that the deficiency in the craniofacial growth in our experiment might be due to the diabetic condition in the DM group as it has been reported that specific changes in bone metabolism are associated with DM. In addition, some of the pathogenic potential, insulinopenia, microvascular bone, dysregulation of mineral metabolism, changes in local factors that regulate bone remodeling, and even an intrinsic disorder related to type 1 diabetes, have been proposed [82, 83]. It is thought that the aforementioned deficiency of the insulin associated with type 1 diabetes may have a direct effect on bone metabolism. It was reported that normal insulin levels exert a direct anabolic effect on bone cells [82]. Multiple osteoblast-like cell lines, expressing the insulin receptor on the cell surface, have a high capacity for insulin binding [84]. Moreover, osteoclast are known to reduce bone resorption in response to insulin stimulation [85]. These findings support the view that insulin in bone can act directly against osteoblasts in combination with the inhibition of osteoclasts [60, 85], and this mechanism of action can be used to explain the delay in the craniofacial growth in STZ-DM. Diabetes has a detrimental effect on osseous turnover due to decreased both osteoblast and osteoclast activities and numbers and, a lower percentage of osteoid surface and osteocalcin synthesis, as well as increased time for mineralization of osteoid [82]. In a separate stage in matrix-induced endochondral bone formation, the influence of diabetes was reported to have a significant impact on the biomechanical behavior of bone. In addition, chondrogenesis and calcification of bone were reduced by 50% in diabetic animals [86]. This was also consistent with our findings that showed a significant reduction in the craniofacial linear measurements of the DM group. In addition, insulin can exert synergistic effects with other anabolic agents on bone, such as parathyroid hormone (PTH) [60, 85]. Type 1 diabetes animal models frequently show the alteration in bone turnover, retarded growth, increased concentration of PTH, and reduced concentration of 1,25-dihydroxyvitamin D [82, 87]. The effects of PTH on the bones are rather complex; PTH stimulates resorption or bone formation depending on the concentration used, the duration of the exhibition, and the administration method [82, 86, 87, 88]. Moreover, 1,25-dihydroxivitamin D, like PTH, belongs to the most important group of bone regulatory hormones. It regulates osteoclastic differentiation from hematopoietic mononuclear cells, and osteoblastic functions and activity [82, 89].

Moreover, insulin may indirectly regulate the increase in the concentration of growth hormone (GH) in serum concentration by direct regulation of the hepatic growth hormone receptor. That would result in abnormalities in the insulin growth factor-1 (IGF-1) in T1DM [90] which consequently might have led to the retarded growth in uncontrolled DM, in our study. In the present study, the mineral appositional rates and bone formation rate in DM group were significantly lower in the most area of periosteal surface in mandible as compared to the control group. These results are in agreement with the previous studies that reported diminished lamellar bone formation in DM rats' femur and may suggesting the putative association between the DM condition and the decreased number and function of osteoblasts [61]. The alveolar crest region was the only region that did not show a significant difference in the mineral apposition rate and the bone formation rate parameters between healthy and DM

groups; this may be attributed to the unique nature of this region exhibiting a highly intensive bone remodeling process especially during the teeth eruption that decreases toward the base of the socket [77]. A significant decrease in bone volume fraction, trabecular thickness, and trabecular numbers was confirmed by micro-CT analysis in DM rats. DM rats also showed a significant increase in the trabecular separation and the trabecular space when compared with the control group. This finding indicated the deterioration of the bone quality in the DM group. These observations are in agreement with other works suggesting that the glycemic levels play an important role in modulating the trabecular architecture especially in mandibular bone [60]. In this context, these results may describe a state of osteopenia in experimental diabetic rats, which might be caused by an imbalance between bone formation and resorption. A histometric evaluation of bone resorption was performed by counting the number of osteoclast cells on the distal surface of the alveolar bone adjacent to the mesio-buccal root of the second molar. These evaluations revealed that the number of osteoclasts was significantly lower in the DM rats than in the controls, in line with the previous studies on DM rats' mandible and long bones [58]. These studies confirm that the decreased rate of bone turnover may be associated with the DM condition. This worsening effect of the structure and dynamic bone formation on mandible might be due to a number of pathogenic potentials such as insulinopenia, bone microangiopathy, impaired regulation of mineral metabolism, alteration in local factors that regulate bone remodeling [57, 83]. However, the adverse effects observed may not be associated with the significant loss of rats' weights observed in the diabetic group starting from day 14 because previous research [57, 60] showed that the mandibular growth was not affected in normal rats supplied with restricted diet and having same pattern of weight loss resembling weight loss pattern observed in DM rats.

3.11. Expected mechanism of type 1 diabetes detrimental effects on tooth

Many investigations focused on the various detrimental effects exerted by the type 1 diabetes on different body organs; however, less attention was paid to the effect of such condition on teeth. A previous study suggested that the diabetic condition may exert detrimental effects on enamel formation [91]. However, that study was conducted on an extremely small sample size of different types of rodents suffering from diabetic conditions that were either genetically induced or drug induced and did not include a proper number of control rats. Thus, it was of an extreme importance to study the detrimental effect of diabetes on tooth structure formation using enough number of experimental animals and to use accurate methods of measurements as those adopted in the our studies. The null hypotheses tested in our previous study were partly accepted because the type 1 diabetes condition adversely affected the enamel and dentine thickness, and the dentine mineral apposition and dentine formation rates; however, there was no significant effect of the type 1 diabetes condition on the enamel and dentine mineral densities.

We have demonstrated that the type 1 diabetes condition induced detrimental changes on the thickness of enamel and dentine. Thus, it could be speculated that the metabolic functions of the ameloblasts and the odontoblasts may be hindered by the elevated blood glucose level associated with the type 1 diabetes condition. It was previously suggested that the type 1

diabetes condition affect ameloblasts and odontoblasts by a mechanism similar to the well-documented mechanism exerted by the type 1 diabetes condition on osteoblasts bone-forming cells due to the similarities between the process of dentine, enamel, and bone development [92]. Moreover, several genetic disorders were found to affect both the osteoblasts and odontoblasts and thus affecting the mineralization process of bone and dentine, respectively [92]. However, in contrast to bone, dentine and enamel do not remodel and are not involved in the regulation of the calcium and phosphate metabolism [93].

It was previously demonstrated that a glucose concentration similar to those observed in poorly controlled diabetic patients inhibited the osteoblast cells from depositing calcium during the mineralization process of the bone matrix [94]. One can speculate that a similar inhibitory effect was exhibited in the current study by the high glucose level on the activities of the odontoblasts and ameloblasts during the enamel and dentine formation. This inhibitory effect of increased glucose level on ameloblasts and odontoblasts was suggested by a previous study that showed that the total calcium content in rat teeth suffering from type 1 diabetes was significantly lower than those of their controls [95]. Another study reported a significant decrease in cultured pulp cells ability to proliferate and decreased mineralized nodule formation upon exposure to high levels of glucose [96]. Another mechanism that might explain the negative effects exerted by the type 1 diabetes condition on odontoblasts and ameloblasts activities may be attributed to the increase in blood glucose level that interferes with the maturation and the proper mineralization of the dentine collagen matrix during the dentine development stages [97]. Previous research work showed that the histological features of the ameloblast and its function might be affected by the increased glucose level associated with the type 1 diabetes condition [98]. Moreover, several clinical observations showed that enamel susceptibility to caries and the incidence of enamel hypoplasia increased in type 1 diabetes patients [99]. Furthermore, it was previously suggested that type 1 diabetes condition may exert a generalized decrease in the metabolic activities of bone cells. All of the aforementioned findings may suggest that the observed harmful effects exerted by the type 1 diabetes condition on enamel and dentine in this study may be a part of a generalized detrimental effect exerted by the diabetic condition on osteoblasts, odontoblasts, and ameloblasts.

4. Conclusion

It is obvious that type 1 diabetes condition significantly affects craniofacial growth, bone formation mechanism, and the quality of the bone formed, which may alter many aspects of planning and treatment of orthodontic patients affected by this globally increasing hormonal disturbance. Moreover, type 1 diabetes condition impairs the proper tooth development and alters the oral environment rendering teeth more susceptible to dental caries. There should be a new strategy for treating orthodontic patients suffering from metabolic disorders specially those disorders having direct and indirect effects on bone growth as the diabetic condition. The orthodontic craniofacial linear measurements were significantly decreased in the type 1 diabetes cases when compared to normal cases. Moreover, greater risks of developing dental caries and possible tooth loss are associated with patients suffering from type 1 diabetes; these

risks may complicate the outcome of orthodontic treatment which is associated with less ability of orthodontic patients to implement proper oral hygiene measures due to increased areas of bacterial biofilm formation around orthodontic brackets. These comprehensive studies carried out on bone and craniofacial growth suggest that planning the treatment in craniofacial region for patients affected with hormonal disorders is more complex procedure than the treatment of normal patients. Up-to-date data also suggest that it is of prime importance to keep close attention to the general systemic condition of these patients and administer the proper hormonal therapy for these patients when needed to avoid any detrimental effects on bone resulting from any hormonal imbalance. Moreover, the results of tooth analysis in experimental type 1 diabetes model showed that the type 1 diabetes condition suppressed the enamel and dentine formation; however, the enamel and dentine densities were not affected. This indicates that diabetic patients may be more susceptible to dental caries and teeth size discrepancies. Type 1 diabetes patients' dental problems should be handled carefully, and their diabetic condition monitoring is of prime importance, especially during early stage of tooth development.

Author details

Ippei Watari[1*], Mona Aly Abbassy[2,3], Katarzyna Anna Podyma-Inoue[4] and Takashi Ono[1]

*Address all correspondence to: ippeiwatari@gmail.com

1 Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Science, Graduate School of Medical and Dental Science, Tokyo Medical and Dental University (TMDU), Tokyo, Japan

2 Department of Orthodontics, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia

3 Alexandria University, Alexandria, Egypt

4 Section of Biochemistry, Department of Bio-Matrix, Graduate School of Medical and Dental Science, Tokyo Medical and Dental University (TMDU), Tokyo, Japan

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