ORIGINAL ARTICLE

EFFECT OF DIABETES MELLITUS ON ORTHODONTIC TOOTH MOVEMENT IN A RAT MODEL

[1]ARFAN UL HAQ
[2]WAHEED-UL-HAMID
[3]HAFIZ SHAKER MAHMOOD
[4]SHABBIR HUSSAIN
[5]SAMEEN IRFAN

ABSTRACT

The objective of this study was to observe bone response in terms of osteoclast count and orthodontic tooth movement under optimal orthodontic force in diabetic and normoglycemic rat model. This experimental study was conducted at Animal House and Histopathology Department, Post Graduate Medical Institute, Lahore. Duration of study was June 2013 December 2013. 44 male wistar rats were included in the study and equally divided into two groups; group 1 (Normoglcemic or NG group) and Group 2 (Experimental Diabetic or EDB group). Type-1 diabetes mellitus was induced by injecting streptozotocin (STZ) in EDB group. Citrate buffer solution was injected in NG group. Maxillary right first molar was moved mesially by applying 10 cN force using closed coil spring. All rats were euthanized on the 21st day after placement of the appliance. The orthodontic tooth movement was recorded by digital vernier caliper. Maxillae of the rats were dissected along with the molar teeth. Serial transverse sections of each maxilla in the interradicular bone at furcation area of first molar distobuccal root of control and appliance side were obtained for quantification of osteoclasts by histomorphometric study. Results showed that mean osteoclast count was significantly more in EDB group as compared to NG group, while no osteoclast was found on the non-appliance control side of both groups. Mean orthodontic tooth movement of rats in EDB group was significantly higher than NG group. It was concluded that Type-1 Diabetes Mellitus result in greater orthodontic tooth movement and increased osteoclasts as compared to normal subjects.

Key Words: *Diabetes Mellitus, Orthodontic Tooth Movement, Osteoclast, Bone Remodeling.*

INTRODUCTION

Type 1 diabetes mellitus results from the body's failure to produce insulin which usually strikes children and young adults. Prevalence rate of diabetes is increasing worldwide and Pakistan is amongst the top ten countries.[1] Chronic hyperglycemia leads to number of complications by damaging different organs including the heart, eyes, kidneys, nerves, bones etc.[2]

[1] **Corresponding Author:** Dr Arfan-ul-Haq BDS, MDS, FCPS (Orthodontics); MCPS (Oper.D) Associate Professor Orthodontics, de,Montmorency College of Dentistry, Fort Road Off Ravi Road, Lahore, Pakistan E-mail: ortho.irfanulhaq@gmail.com Cell: 0333-4207669
[2] Prof Waheed-ul-Hamid, BDS, MS, M.Orth, MCPS (Orthodontics) Principal/ Head of Orthodontic Department, de,Montmorency College of Dentistry, Lahore
[3] Dr Hafiz Shaker Mahmood, BDS, MDS, MCPS (Oral & Maxillofacial Surgery) Senior Registrar, Department of Oral & Maxillofacial Surgery, King Edward Medical University, Lahore
[4] Dr Shabbir Hussain, BDS, Senior Demonstrator, Department of Orthodontics, de,Montmorency College of Dentistry, Lahore, Pakistan
[5] Dr Sameen Irfan, BDS, Clinical Observer, Irfan Dental Clinic, 3-Race Course Road, Lahore

Received for Publication: February 23, 2016
Approved: March 8, 2016

Type 1 diabetes mellitus adversely affects the bone health status resulting in decreased bone mass.[3] One of the major mechanisms which lead to reduced bone mass is decreased bone formation and osteopenia due to microangiopathy.[4] Hyperglycemia in diabetic patients inhibit the proliferation and differentiation of periodontal cells, delayed periodontal regeneration and healing due to decreased function of transcription factor (Runx)-2, osteocalcin and osteopontin expression.[4,5]

The assessment of bone response to orthodontic forces in diabetic wistar mice showed a significant decrease in number of osteoclast as observed in periodontal cortex of the dental alveolus in experimental diabetic group.[6] It is experimentally proved that Orthodontic tooth movement is an inflammation-like process, achieved by remodeling process of alveolar bone, periodontal ligament and sometimes cementum.[7,8]

Appropriate Orthodontic force when applied, tooth moves in the alveolar bone. It is emphasized

that orthodontic force level is related to the surface area of the root of the tooth for an adequate biological response.[9] Rat molar with fifty times smaller size than human molar, requires less force to move but general shortcomings exist pertaining to level of applied force on tiny molar in experimental setup of rat studies.[10] In various animal studies, 35 to 120 cN force level was used.[6,11] However it is clarified that forces exceeding the optimal level will impede the recruitment and differentiation of cells and cause hyalinization and both these processes hamper tooth movement.[9]

It is pointed out that important physiological process of tooth movement can be derailed by diabetes as orthodontic tooth movement depends upon balanced alveolar bone remodeling and diabetes might affect this process.[12,13] Alteration in the bone metabolism is a common observation in the long-term complications of diabetic patients and even in treated patients, However, little data is available about how diabetes mellitus affects orthodontic tooth movement.[6,11]

The objective of this study was to analyze bone response in terms of osteoclast count and magnitude of orthodontic tooth movement in Normoglycemic and Experimental Diabetic rats under optimal orthodontic force application.

METHODOLOGY

This animal experimental study was conducted at Animal House and Histopathology Department, Post-graduate Medical Institute, Lahore. Duration of study was June 2013 December 2013. 44 wistar male rats with body weight 200 gm aged 10-12 weeks having fasting blood glucose 80 mg/dl were included in the study. Rats suffering from any disease or allocated for other study were excluded. These rats were randomly divided into two groups. Each group comprising twenty two rats with different color coding; Group 1 (Blue): Normoglcemic (NG) group and Group 2 (Red): Experimental Diabetic (EDB) group. Each rat in both groups was allocated number 1-22. Rats in EDB group were kept fasting for twelve hours then rendered type-1 diabetic by single dose intraperitoneal injection of 80 mg/kg body weight streptozotocin (STZ) freshly prepared in ice-cold 0.5 mol/l citrate buffer solution (pH 4.5). Rats with blood glucose level $\geq$ 300 mg/dl were considered as diabetic otherwise injection was repeated. Citrate buffer solution was injected in NG group. Blood sample was taken from the rat tail tip and random blood glucose level estimated with glucometer regularly. Body weight was measured with rat weighing machine.

The rats were anesthetized with the cocktail of ketamine 80 mg/kg body weight and xylazine 10 mg/kg body weight. Using split mouth design orthodontic appliance was placed only on right side of the rat maxilla while left side without appliance was taken as control. Maxillary right first molar was moved mesially by applying 10 cN optimal force with nickel titanium (Ni-Ti) closed coil spring tied between incisor and molar teeth and force measured by tension gauge.

All rats were euthanized with the overdose of pentobarbital on the 21st day after placement of appliance. The orthodontic tooth movement was recorded with the help of digital vernier caliper (with measuring accuracy of 0.01mm). D1 was the relative separation between the mesial occlusal pits of maxillary first and second molars before placement of appliance and D2 after the application of orthodontic force at 21st day in millimeters. The orthodontic tooth movement was the difference between D2 and D1.

Maxilla of the rats were dissected along with the molar teeth. It was divided into right and left halves and trimmed around 1st molar tooth. Fixation and decalcification were done with 10% formaline and nitric acid. After conventional processing of the specimen it was embedded in paraffin mould and sectioned with the help of microtome. Serial transverse sections (6 micron thick) of each maxilla in the interradicular bone at furcation area of mesial pressure side of first molar disto-buccal root of control and the appliance side were obtained. Three sections (S1, S2 and S3) of each animal were selected for histomorphometric study. After staining with hematoxylin and eosin, number of osteoclasts (multinuclear cells in the resorption lacunae close to the bone surface) were quantified under × 40 magnifications in five consecutive microscopic fields by two histopathologist.

Statistical Analysis

The data was entered and analyzed by using SPSS version 18. The results of the study were expressed as mean ± standard deviation. One way analysis of variance (ANOVA) was applied to calculate any significance among the groups. In case of any difference;

TABLE 1: COMPARISON OF BODY WEIGHT AND FASTING BLOOD SUGAR IN EXPERIMENTAL DIABETIC RAT (EDB) WITH THEIR NORMOGLYCEMIC (NG)

Subjects	Initial weight (gm)	Final weight (gm)	Final fasting blood sugar (mg/dl)
Normal (NG) (22)	203.86±12.61	272.36±20.77**	80.00±1.18
EDB (22)	205.41±8.16	214.50±21.93**	314.68±7.77

ANOVA= 74.774 P-value= 0.000 (significant)

TABLE 2: COMPARISON OF MEAN OSTEOCLAST COUNT OF ALL SECTIONS OF RATS IN NORMOGLYCEMIC (NG) AND EXPERIMENTAL GROUP (EDB)

Means of all sections	NG (22)	EDB (22)
Non appliance or control side	0±00	0±00
Appliance side	2.94±0.42	7.15±0.63
P value	<0.001	<0.001

ANOVA= 592.60 P-value < 0.001 (significant)

TABLE 3: COMPARISON OF ORTHODONTIC TOOTH MOVEMENT OF RATS IN EDB AND NG GROUPS

Subjects	OTM (mm)
NG (22)	0.34±0.07
EDB (22)	0.78±0.09
P value	<0.001

ANOVA= 271.201 P-value = 0.000 (significant)

post-hoc Tukey test was used for pair wise comparison among the groups. A value of $p \leq 0.05$ was considered statistically significant.

RESULTS

Comparison of body weight and fasting blood sugar in experimental diabetic rat (EDB) with their normoglycemic (NG) is tabulated (Table 1). It was observed that final body weight of both normoglycemic and experimental diabetic rat was significantly increased as compared to their initial body weight. On the other hand the final fasting blood glucose level was significantly increased in EDB group as compared to their initial fasting blood glucose level.

Comparison of mean osteoclast count of all sections of rats in control and appliance side is tabulated as Table 2. It was observed that the mean osteoclast count of appliance side of EDB group was significantly increased as compared to counts of normoglycemic and control side. Comparison of orthodontic tooth movement (OTM) in mm of EDB and NG groups is tabulated in Table 3. It is observed that orthodontic tooth movement of experimental rat (EDB) is significantly more than the tooth movement of normoglycemic.

DISCUSSION

Total 44 wistar rats were included in this experiment. Initial weight of all the rats of each group was nearly similar and the difference among all the groups was insignificant. The initial weight of rats in NG, EDB groups was 204.55±11.63, 205.41±8.16 grams and increased with the advancing age to 279.82±14.66, 214.50±21.93 grams respectively. Comparison of weights between two groups was statistically significant and EDB group has less increase in weight as compared to NG group at the end of procedure. Another study also found that the mean weight of all studied rats was increased. This may be due to the fact that experimental rats were not subjected to any metabolic disorder.[14] however another study carried out to evaluate influence of stress on orthodontic tooth movement indicated that stress group experienced loss of weight during the experiment due to interference with the normal body physiology.[15]

In the current study fasting blood sugar level of NG and EDB groups were 73.95±1.79 and 314.68±7.77 respectively. The glycemic status of EDB rats was remained above 300 mg/dl during the experimental period. According to a study conducted on experimental diabetic rats, blood glucose level remained greater than 250 mg/dl.[6] While the blood sugar levels of diabetic mice were 483 ± 24.4 mg/dl in the study of another group of workers.[11] This variation of fasting blood sugar level in diabetic animals in various studies may be due to the use of different doses of streptozotocin to induce diabetes and type of animals used.

The results of present study revealed the effect of type-1 diabetes mellitus in terms of greater magnitude of orthodontic tooth movement and increased osteoclasts count in EDB rats as compared to NG rats on appliance side. The findings of current study regarding up-regulation of osteoclasts number and greater magnitude of orthodontic tooth movement in type-1 diabetes mellitus are consistent with previous studies.[4,11,16] It is proposed that the patients undergoing orthodontic therapy can have the variation in the biological process involved in tooth movement.[17] However, it is still controversial that how this metabolic disorder can influence orthodontic tooth movement.[11]

In the present study, orthodontic load application resulted in bone remodeling and emergence of osteoclasts. Number of osteoclasts was quantified in the histological sections on the mesial pressure side of disto-buccal root of right maxillary first molar near the furcation level. The mean osteoclast count on appliance side in NG and EDB groups showed that EDB group has more osteoclast count as compared to NG group with a significant difference ($p<0.05$). On the control or non-appliance side of each group no osteoclast was seen. Our study is in line with a study by Braga[11] who observed that greater number of TRAP-Positive osteoclasts were identified on the appliance side in streptozotocin induced diabetic mice as compared to normoglycemic mice. He also found that Chemokine and cytokine expression was enhanced in diabetic mice. The placement and activation of orthodontic appliance resulted in increased level of osteoclastic

markers in periodontal tissue which were greater in diabetic mice as compared to normal subjects.[11] A group of workers also reported that diabetes mellitus results in exacerbation of osteoclasts and amplification of bone resorption phenomenon.[4] In a previous study[16] it was observed that drug induced diabetic mice with high dose of streptozotocin (repeated dose of 60mg/kg) leads to increased severity of diabetes mellitus and enhanced number of osteoclasts as compared to the low dose of streptozotocin introduced diabetic group (repeated dose of 40mg/kg). It is proposed that high dose of streptozotocin injection exhibited an increase in RANKL/OPG ratio and bone cathepsin-k, which led to increased osteoclastic activity. The findings of these studies are consistent with current study in terms of increase in the number of osteoclast in diabetic subjects.

In this study, it is demonstrated that alteration in bone remodeling process in diabetic rat resulted in greater amount of orthodontic tooth movement, after mechanical loading as compared to normoglycemic rats. The mean orthodontic tooth movement of rats in NG and EDB were 0.34±0.07 and 0.78±0.09 mm respectively. The difference was significant and EDB group has more orthodontic tooth movement as compared to NG group. A study[11] on mice also reported increase in the number of TRAP-positive cells, enhanced bone resorption and greater amount of orthodontic tooth movement in streptozotocin induced diabetic mice as compared to normoglycemic after mechanical loading. It is proposed that balanced alveolar bone remodeling mechanism in response to optimal orthodontic force is necessary for desired tooth movement and health of bone is a primary factor to achieve this planned outcome.[6] Factors such as gender, periodontal status, force level, medications, certain diseases and even laser therapy can affect this biological mechanism.[18] Diabetes Mellitus is also one of the factors causing variation in the bone remodeling process, involved in orthodontic tooth movement.[11] A study also found that diabetes mellitus has deleterious effects on bone and periodontal health resulting in unbalanced remodeling cycle.[19]

In this experiment an orthodontic appliance made up of Ni-Ti close coil spring was used which delivered continuous optimal force to move the molar tooth mesially. In more than 25% of studies on orthodontic tooth movement in rats, elastics have been used to deliver the forces. The forces generated by elastics degenerate rapidly and needs to be replaced for reactivation. This short coming may be overcome by using Ni-Ti close coil spring delivering a light continuous force for a longer period of time.[20]

A major controversial point is that whether results obtained from the animal experiments are applicable to human beings or not. Morphology and physiology of human and rat periodontal ligament and alveolar bone needs to be considered. Rat alveolar bone is dense and it has no osteons and marrow spaces while there is less amount of osteoid tissue along the bone surface of alveolus as compare to humans.[21] In few studies, it has been reported that acid mucopolysaccaride is less in the rat bone. In human beings, bone tissue controls the balance of calcium while in rats it is controlled by intestinal absorption of calcium. Histological studies have revealed that periodontal ligament and the surrounding structures are structurally different from human beings.[22,23] During normal tooth eruption and after application of force for the purpose of tooth movement, tissue development and changes are faster from that of humans but the principle process involved is same for both.[23] Further studies on human patients with Type-1 diabetes mellitus are recommended to evaluate orthodontic tooth movement under optimal forces in Pakistani population.

CONCLUSION

It is concluded that type-1 Diabetes Mellitus results in greater Orthodontic tooth movement and increased osteoclast count as compared to normal subjects under optimal force level.

REFERENCES

1 Zafar J, Bhatti F, Akhtar N, Rasheed U, Bashir R, Humayun S et al. Prevalence and risk factors for diabetes mellitus in a selected urban population of a city in Punjab. J Pak Med Assoc 2011; 61 (1): 40-47.

2 Mealey BL and Oates TW. Diabetes mellitus and periodontal diseases. J Periodontol 2006; 77 (8): 1289-303.

3 Saller A, Maggi S, Romanato G, Tonin P. and Crepaldi G. Diabetes and osteoporosis. Aging Clin Exp Res 2008; 20 (4): 280-89.

4 Wongdee K and Charoenphandhu N. Osteoporosis in diabetes mellitus: Possible cellular and molecular mechanisms. World J Diabetes 2011; 2 (3): 41-48.

5 Kim HS, Park JW, Yeo SI, Choi BJ and Suh JY. Effects of high glucose on cellular activity of periodontal ligament cells in vitro. Diabetes Res Clin Pract 2006; 74 (1): 41-47.

6 Villarino ME, Lewicki M and Ubios AM. Bone response to orthodontic forces in diabetic Wistar rats. Am J Orthod Dentofacial Orthop 2011; 139 (4 suppl): 76-82.

7 Bhosale V, Hazarey P and Halli RC. Histochemical Analysis of Acid-phosphatase Activity Incident To Orthodontic Tooth Movement In Albino Rats-An Experimental Study. WebMed Central Orthodont 2011; 2: WMC001653.

8 Liang Y, Zhou Y, Jiang T, Zhang Z, Wang S and Wang Y. Expression of LIF and LIFR in periodontal tissue during orthodontic tooth movement. Angle Orthod 2011; 81 (4): 600-08.

9 Ren Y, Maltha JC, Van't Hof MA and Kuijpers-Jagtman AM. Optimum force magnitude for orthodontic tooth movement: a mathematic model. Am J Orthod Dentofacial Orthop 2004; 125 (1): 71-77.

10 Hamann C, Kirschner S, Gunther KP and Hofbauer LC. Bone, sweet bone-osteoporotic fractures in diabetes mellitus. Nat Rev Endocrinol 2012; 8 (5): 297-305.

11 Braga SMG, de Albuquerque Taddei SR, Andrade I, Queiroz-Junior CM, Garlet, G.P et al. Effect of diabetes on orthodontic

tooth movement in a mouse model. Eur J Oral Sci 2011; 119 (1): 7-14.

12 Janghorbani M, Van Dam RM, Willett WC and Hu FB. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. Am J Epidemiol 2007; 166 (5): 495-505.

13 Feng X and McDonald JM. Disorders of Bone Remodeling. Annu Rev Pathol 2010; 6: 121-45.

14 Kohno T, Matsumoto Y, Kanno Z, Warita H, Soma K. Experimental tooth movement under light orthodontic forces: rates of tooth movement and changes of the periodontium. Journal of orthodontics 2002; 29 (2): 129-36.

15 Mirzakouchaki B, Firoozi F and Shahrbaf S. Effect of psychological stress on orthodontic tooth movement in rats. Med Oral Patol Oral Cir Bucal 2011; 16 (2): 285-91.

16 Motyl K J and McCabe LR. Streptozotocin, type I diabetes severity and bone. Biol Proced Online 2009; 11: 296-315.

17 Bensch L, Braem M, Acker KV and Willems G. Orthodontic treatment considerations in patients with diabetes mellitus. Am J Orthod Dentofacial Orthop 2003; 123: 74-78.

18 Youssef M, Ashkar S, Hamade E, Gutknecht N, Lampert F and MIR M. The effect of low-level laser therapy during orthodontic movement: a preliminary study. Lasers in medical science 2008; 23: 27-33.

19 Demmer RT, Holtfreter B, Desvarieux M, Jacobs JR DR, Kerner W, Nauck M et al. The influence of type 1 and type 2 diabetes on periodontal disease progression: prospective results from the Study of Health in Pomerania (SHIP). Diabetes Care 2012; 35 (10): 2036-42.

20 Ren Y, Maltha JC and Kuijpers-Jagtman AM. The rat as a model for orthodontic tooth movement a critical review and a proposed solution. European Journal of Orthodontics 2004; 26 (5): 483-90.

21 Reitan, K. Clinical and histologic observations on tooth movement during and after orthodontic treatment. Am J Orthod 1997; 53 (10): 721-45.

22 Romanos G and Bernimoulin J. [Collagen as a basic element of the periodontium: immunohistochemical aspects in the human and animal. 1. Gingiva and alveolar bone]. Parodontol 1990; 1 (4): 363-75.

23 Romanos G, Schroter-Kermani C and Bernimoulin J. [Collagen as a basic element of the periodontium: immunohistochemical aspects in the human and animals. 2. Cementum and periodontal ligament]. Parodontol 1991; 2 (1): 47-59.

24 Rygh P. Hyalinization of the periodontal ligament incident to orthodontic tooth movement. American journal of orthodontics 1976; 70 (3): 269-81.

CONTRIBUTION BY AUTHORS

1 Arfan ul Haq:	Corresponding author, article writing and final compilation.
2 Waheed-ul-Hamid:	Supervisor and guidance.
3 Hafiz Shaker Mahmood:	Statistical analysis and results compilation.
4 Shabbir Hussain:	Data collection.
5 Sameen Irfan:	Final article compilation and grammatical/spelling editing.