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Effect of Insulin Treatment on Orthodontic Tooth Movement and Osteoclast Count in Diabetic Rats

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ABSTRACT

Objectives: To estimate osteoclast count and orthodontic tooth movement in a group of insulin treated diabetic rats. **Study Design:** Animal experimental study. **Setting:** Animal House and Histopathology Department, Post-graduate Medical Institute, Lahore. **Duration:** One year, June 2013- June 2014. **Sampling Technique:** Simple random allocation **Methodology:** Total 44 male wistar rats were selected and equally divided into Normoglycemic and Insulin Treated Diabetic groups. Type-1 diabetes mellitus was induced by injecting streptozotocin then treated with Insulin injections. Citrate buffer solution was injected in normoglycemic group. The rats were anesthetized with cocktail of ketamine and xylazine injections. Using split mouth design orthodontic appliance was placed only on right side of maxilla while left side was kept as control. Maxillary right first molar was moved mesially by applying 10 cN force. All rats were euthanized on 21st day and orthodontic tooth movements were recorded using digital vernier calliper. Serial transverse sections of dissected maxilla in the interradicular bone at furcation area of first molar distobuccal root were obtained for quantification of osteoclasts by histomorphometry. **Results:** Mean osteoclast count in normoglycemic group was 2.94±0.42 and 2.65±0.36 in insulin treated diabetic group with significant difference, while no osteoclast found on control side. Mean orthodontic tooth movement in normoglycemic group was 0.34±0.07 mm while 0.34±0.06 mm in insulin treated diabetic group with non-significant difference. **Conclusion:** Insulin therapy reversed the diabetic condition to the same level as that of normal subjects.

Keywords: Type-I diabetes mellitus, Orthodontic Tooth Movement, Insulin therapy.

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INTRODUCTION

Type-1 Diabetes Mellitus (DM) is caused by the immune destruction of the beta cells of Langerhans of pancreas and Insulin secretion is gradually diminished. About seven million people are estimated to be diabetic in Pakistan and at present Pakistan stands at eighth number in the world for this disease prevalence. By the year 2025, the figure may rise from 7 to 15 million and the position may go to fourth number in the world.[1]

It is suggested that type-1 diabetes mellitus contribute to bone loss through increased cathepsin-K and tartrate-resistant acid phosphatase expression as well as reduced osteocalcin in osteoblasts resulting in increased bone resorptive activity and decreased bone formation.[2] Several studies have demonstrated a positive role between bone mineral density and insulin directly or indirectly by increasing hepatic insulin like growth factor (IGF-1). Osteoblast like cells have the insulin receptors and it stimulates proliferation of these cells. In diabetic rat, reversal of the diabetic state by insulin treatment results in morphological findings similar to those of normoglycemic rat.[3] The assessment of bone response to orthodontic forces in experimental diabetic wistar mice treated with insulin showed a significant decrease in number of osteoclast in periodontal cortex of the dental alveolus.[4]

Optimal force is based on proper mechanical principles, which enables the orthodontist to move teeth without traumatizing dental or periodontal tissues and avoiding damage to the roots of teeth.[5] Orthodontic tooth movement is an inflammation-like process characterized by process of osteoclastogenesis and osteogenesis. Appropriate orthodontic force when applied, tooth moves in the alveolar bone. Controlled and standardized force system is coherent with adequate biological response and minimal tissue damage.[6]

Due to the high prevalence of DM, the probability of the orthodontic treatment in such patients is also very high. Therefore, it is the responsibility of an orthodontist as well as dentist to have a basic knowledge of the signs and symptoms of DM.

Patients having good glycemic control can undergo orthodontic treatment whereas treatment of uncontrolled diabetics is not indicated.[7,8] Periodontal health should be monitored during treatment with proper oral hygiene instruction and appointments should be planned in the morning following insulin injection and breakfast. Orthodontic considerations include delaying orthodontic treatment when diabetes is poorly controlled. [9]

Objective: The objective of this study was to analyze bone response in terms of osteoclast count and magnitude of orthodontic tooth movement in normoglycemic and insulin treated diabetic rats using optimal orthodontic forces. This study will be helpful for orthodontists while treating patients suffering from type-I diabetes mellitus under insulin therapy.

METHODOLOGY

Study Design: Animal experimental study. Split-mouth design

Place of Study: Animal House and Histopathology Department, PGMI, Lahore.

Period: One year, June 2013 - June 2014.

Sampling technique: Simple Random allocation

Inclusion criteria:

1. Male Wistar rats.
2. 10 to 12 weeks age
3. 200 ± 20 gms weight
4. Fasting blood glucose level 80±10 mg/dl.

Exclusion criteria:

1. Rats specimen for other studies.
2. Rats suffering from any disease.

Data Collection Procedure

Total 44 male wistar rats were selected according to inclusion criteria and randomly divided into two groups. Each group comprising twenty two rats with different color coding; Group 1 (Blue): Normoglycemic (NG) group and group 2 (Green): Insulin Treated Diabetic (ITDB) group. Each rat in both groups was allocated number 1-22. Rats for ITDB 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 ≥ 300 mg/dl were considered diabetic otherwise injection was repeated. These Experimentally rendered diabetic rats were treated with Insulin injection. 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 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 40X magnifications in five consecutive microscopic fields by two histopathologists. Average values for all calculations were taken for further analysis.

Data 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; 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 insulin treated diabetic rat (ITDB) with their controls (NG) is tabulated (Table 1). It was observed that final body weight of both control and insulin

treated 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 ITDB 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 in table 2. It was observed that the mean osteoclast count of appliance side of ITDB was significantly decreased as compared to NG.

Comparison of OTM of rats in ITDB and NG is tabulated in table 3. It was observed that there is no change in orthodontic tooth movement of insulin treated diabetic rat (ITDB) when compared with tooth movement of NG group.

Table 1: Comparison of body weight and fasting blood sugar in experimental insulin diabetic rat (ITDB) with normoglycemic (NG)

No of parameters in parenthesis		Values expressed as mean±SD	
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
ITDB (22)	204.55±11.63	279.82±14.16**	314.68±7.77**

ANOVA= 74.774 P-value= 0.000 (significant)

Table 2: Comparison of mean osteoclast count in normoglycemic (NG) and insulin treated diabetic group (ITDB) as well as control side

Means of all sections	NG (22)	ITDB (22)
Non-appliance or control side	0±00	0±00
Appliance side	2.94±0.42	2.65±0.36
P value	<0.001	<0.001

ANOVA= 592.60 P-value < 0.001 (significant)

Table 3: Comparison of orthodontic tooth movement (OTM) of rats in ITDB and NG

Subjects	OTM (mm)
NG	0.34±0.07
ITDB	0.34±0.06

ANOVA = 271.201 P-value = 0.000 (significant)

DISCUSSION

This study was conducted on 44 male wistar rats to evaluate orthodontic tooth movement and osteoclast count during orthodontic treatment under optimal orthodontic forces**.** Certain factors can affect the biological process of tooth movement such as gender, periodontal status, force level, medications, certain diseases and even laser therapy. [10] Diabetes mellitus is chronic disease leading to disturbed oral health status especially compromised periodontal health.[11,12,13] Before starting discussion and establishing the conclusion, it should be pointed out that only few studies have been carried out to demonstrate the role of Insulin therapy in type-1 diabetes mellitus on bone remodeling and orthodontic tooth movement. A study conducted on diabetic rats showed an increased orthodontic tooth movement and osteoclast count.[14] It has been demonstrated that administration of insulin in diabetic rats normalizes the altered bone metabolism and there is a positive correlation between the bone mineral density and the dose of insulin in diabetics.[15] The results of this study showed that insulin therapy in insulin treated diabetic group resulted in nearly the same magnitude of orthodontic tooth movement and osteoclasts number as compared to normoglycemic group. This reversal of altered condition to normalcy is in line with the results obtained by Villarino et al. [3] Braga et al revealed from histopathological statistics of mice study that treatment of streptozotocin induced diabetes mellitus with insulin resulted in reversal of osteoclast number and orthodontic tooth movement to normalcy.[6]

According to our study mean osteoclast count on appliance side of insulin treated diabetic group was significantly decreased as compared to counts of controls. It is reported that high insulin levels are associated with increased bone metabolism-related gene expression in mice, which subsequently may increase bone mass density and improve trabecular micro-architecture.[16]

Orthodontic tooth movement is achieved by the remodeling of alveolar bone in response to mechanical loading. Present study revealed that there is no change in orthodontic tooth movement of insulin treated diabetic rat when compared with tooth movement of normoglycemic. According to another study, orthodontic appliances were placed in normoglycemic and insulin-treated diabetic mice. The Histomorphometric analysis and PCR of periodontium revealed that mice treated with insulin resulted in morphological findings similar to those of normal mice. Their study concluded that the diabetic state up-regulates osteoclast migration and down-

regulates osteoblastic differentiation, resulting in greater orthodontic tooth movement.[5] However the regulation of blood glucose level through insulin therapy decreased these abnormal responses to orthodontic force application.[13] Present study is in accordance with these studies showing that insulin treatment improves the condition leading to orthodontic tooth movement and osteoclast count similar as normoglycemic rats.

This animal study revealed that orthodontic tooth movement in diabetic rat treated with insulin does not vary significantly from that noted in healthy rats. The present study provides the scientific evidence for the contraindication of orthodontic treatment in patients with uncontrolled diabetes mellitus, as orthodontic loading may produce undesired effects. Diabetic patients can receive orthodontic therapy after normalization of metabolic status. Light orthodontic forces are recommended even when the blood glucose level is well controlled in diabetic patients.

CONCLUSION

It is concluded that insulin therapy reversed type-1 diabetes mellitus to nearly the same level as normal and there was no difference of orthodontic tooth movement as well as osteoclast count between normoglycemic and insulin-treated diabetic mice under optimal force level.

Conflict of Interest: This study was conducted by authors at their own interest and there is no conflict of interest among authors and any person/ institution and organization.

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