INTRODUCTION

The teeth, mouth and face have held seemingly intrinsic fascination for mankind, since time immemorial. In the twentieth century, concepts of dentistry revolved around prevention of dental diseases based on research observation. Trauma to erupted deciduous teeth, as well as jaw fracture and surgery can damage the permanent successive tooth germ. Many clinical studies on the prognosis of traumatized tooth germ have reported various post traumatic phenomenon such as failed amelodentinogenesis, hypo calcification, narrow pulp chamber, disturbed root development, odontome formation, ankylosis and failed eruption.

There are very few experimental studies on the relationship between time of trauma and its effects on the developmental stages of tooth germ.

Therefore, the present study was designed to evaluate the effect of mechanical trauma to the tooth germ of Wistar rats at various developmental stages by light microscopy.

MATERIALS AND METHODS

The 7-day-old five new born Wistar rats were obtained from the Animal Experimental Research Laboratory of Madras Medical College and Research Institute, Chennai for this experimental study. When this study was conducted as part of post graduation dissertation, animal clinical registration number was not mandatory, however, we ensure that proper animal handling and good animal care was given in the entire study. The study was approved by Animal Ethics Committee of the Institution.

Upper right 1st molar region were used for experiment and left side of the same animal as control. Under chamber induction anesthesia method rats were put in closed 9 x 4 inches jar containing 3 paper towels saturated with 20 ml of di-ethyl ether until loss of righting reflex (average exposure time of righting reflex was 30 seconds). Dental K file (No.55 Dentsply Maillefer, Ballagues, Switzerland) was inserted through the elevated bulge, the approximate region of the underlying 1st molar tooth germ at an angle of 45 degree with hand force on 7th postnatal day.

The animals were sacrificed by cervical dislocation immediately
on the next day of trauma that is on 8th day, 10th day, 13th day, 19th day and 42nd day for histopathological evaluation. Since, the tooth on 42nd day was not erupted into the oral cavity on the experimental side the maxilla is divided into two halves antero-posteriorly and exposed to in vitro radiograph (65 kvp, 8 mA, 0.2 sec, intraoral peri-apical speed E double emulsion Kodak film, intraoral radiographic machine-Confident Ltd, India)

After sacrificing the animals by cervical dislocation right and left maxilla were dissected out immediately and fixed in 10% buffered formalin for 2 days. Then the specimens were washed well in water and decalcified in 25% formic acid solution for 1 to 4 days.

After complete decalcification, specimens were washed in running water, dehydrated using ascending grades of isopropyl alcohol, cleared using xylene and embedded in paraffin. Mesiodistal sections were made about 5 microns thick and stained using Harris hematoxylin and Eosin, mounted with DPX (Clinar, Glaxo Smithkline Pharmaceuticals Ltd. India) and covered with 18 mm cover glass (Blue Star Ltd, India.). Sections were examined under light microscope at 4 ×, 10 ×, 40 ×, 100 ×, magnifications (Olympus Pvt Ltd, India).

The radiographic findings of control side on the 42nd post natal

| Table 1: Histopathological findings |
|------------------------------------|
| **Post natal day** | **Control side** | **Experiment side** |
| 8th Day | Enamel shows maturation, well formed dentin with odontoblasts and normal pulp in 1st molar. Root Formation is seen. 2nd molar shows normal tooth development | Though the 1st molar shows three cuspid patterns, the tooth size is reduced. Mesial cusp appears rudimentary. Tooth shows disturbed enamel and dentin formation. Pulp tissue continued with periodontal ligament and alveolar bone. The tooth shows arrested crown and tooth development. 2nd molar appears normal. Tooth size is reduced. Root formation is arrested. 2nd molar appears normal. |
| 10th Day | 1st molar shows normal tooth development. Enamel shows process of maturation. Dentin and pulp appears normal. 2nd molar shows normal tooth development | Ulceration of oral mucosa. 1st molar is pushed to adjacent tooth (2nd molar) and it appears smaller in size. Tricuspid pattern is altered. 2nd molar mesial cusp is compressed due to impingement of adjacent 1st molar. |
| 13th Day | 1st molar shows normal tooth development. Enamel shows complete maturation. Dentin and pulp appears normal. 2nd molar shows normal tooth development | 1st molar tooth germ shows disintegration and exfoliation of remnants of calcified tooth structure. Abscess formation is seen in the deeper region. 2nd molar shows tricuspid pattern. Mesial cusp appears smaller in size. |
| 19th Day | 1st molar shows normal tooth development. Enamel shows complete maturation. Dentin and pulp appears normal. Evidence of root formation is seen. Tip of central cusp erupting into oral cavity. 2nd molar shows normal development of enamel, dentin, pulp. Root formation is seen. | There is a failure of eruption of 1st molar tooth in the area of tooth germ, calcified masses suggestive of enamel, dentin, pulp and cementum are irregularly arranged. Pulpal tissue is seen in between the calcified masses. Empty spaces adjacent to the dentin like areas are suggestive of places occupied by mature enamel, which are removed during the process of demineralization. The structure with the conglomeration of the dental tissue is suggestive of “Complex Odontome”. 2nd molar |
| 42nd Day | 1st molar tooth germ shows almost complete root formation. Matured enamel is demineralized by the action of formic acid and fully erupted crown in the oral cavity. Dentin and pulp are normal. 2nd molar shows normal pattern of tooth formation and eruption. | |
day revealed eruption of first, second and third molars with definite morphology of individual teeth with corresponding radio opaque/radiolucent in enamel, dentin and pulp, respectively [Figures 1-6].

Radiograph of experimental side on 42nd post natal day showed absence of 1st molar tooth. In the area of 1st molar tooth germ, calcified conglomerated mass suggestive of complex Odontome is seen. 2nd and 3rd molar are erupted normally [Figures 7 and 8].

Histopathological findings of control and experimental side are described in Table 1.

DISCUSSION

Experimental research is done to test to various hypothesis. Though, the results cannot be applied completely to human systems, existing knowledge could be modified or expanded. Experimental studies with Wistar rats have been done in many fields of dentistry. The dentition of Wistar rats are
monophyodont. The dental formula is I 1/1 C 0/0 M 3/3. The incisor erupts about 10th postnatal day, the molars on the 19th (1st molar), 22nd (2nd molar) and 35th to 40th (3rd molar) day. By 6 weeks the entire set is in use. The size of the molars decreases from 1st molar to 3rd molar. 1st molar has five roots, 2nd molar four, 3rd molar three in the upper jaw. In the lower jaw, 1st molar has four roots, 2nd molar three and 3rd molar has three roots.[3] The present study on the effect of mechanical trauma on the tooth germ of rat molars at 7th post natal day revealed interesting observations.

Various kinds of disturbances in hard tissue formations can occur as a result of trauma to the tooth germ. The relation between the trauma to the deciduous tooth and its effects on successive permanent tooth has been extensively studied.[2,4]

Andreason et al.[2,4] have classified the clinicopathologic entities of developmental disturbances into the following categories. White or yellow brown discoloration of the enamel, horizontal enamel hypoplasia, crown dilacerations, odontome like malformation, root duplication, vestibular root angulation, lateral root angulation, partial or complete arrest of root formation, sequestration of entire tooth germ, ectopic premature or delayed eruption or impaction.

The trauma in the present study was different in genesis that encountered in the dental clinic. The nature and sequelae of trauma were, however, similar to those involved in the intrusion of the deciduous teeth in their permanent successors. Since the traumatic force was intrusive to the tooth germ, the changes are expected to the enamel discoloration defects, hypoplasia and disturbance in amelogenesis, failure of root formation and disturbance in eruption.[21]

The effect of trauma in this study varied from disturbances in enamel and dentin formation, microdontia, arrested crown and root development, impaction of first molar tooth, disintegration and exfoliation of the remnants of calcified tooth structure.[2,4] Abscess formation is seen in deeper regions. One of the tooth failed to erupt and the radiograph showed conglomeration mass, suggesting complex odontome and it was confirmed histopathologically. This radiographic finding was not reported in any other study.

Experimental trauma to the tooth germ with a needle in the rat molar has been studied previously by Levy.[5] He studied the effect of trauma on the right 1st molar tooth germ of four day old 43 Sprague–Dawley rats. He observed the results immediately after the trauma in three rats, 36 days after the trauma in the rest of the animals. Immediately after the trauma there were signs of haemorrhage, strands of ameloblast and odontoblast in various stages of differentiation, remnants of dentin and enamel. In the second group in which the rats were killed, 36 days later the mandible were removed the teeth were observed clinically and histologically. Gross finding was enamel hyperplasia in 21 animals, 4 showed impacted first molar with enamel hypoplasia, regions of reparative dentine were seen where all the enamel was lacking. Region of ankylosis to the adjacent alveolar bone were found. The pulp chamber contained numerous vascular thrombi, excess of cellular cementum were seen along the roots. In one case, there was a calcified mass surrounded by a dense fibrous connective tissue capsule. This mass histologically consists with the appearance of complex composite odontome.

The experimental study of Taniguchi et al.[6] in developing tooth germ of Sprague–Dawley rats with a dental k file showed localized enamel hypoplasia as a direct sequelae of trauma to the tooth germ, dislocation results in ankylosis as an indirect effect of trauma.

Odontomes are considered to be hamartomas rather than a true neoplasm. They chiefly consist of enamel and dentin with variable amount of pulp and cementum when fully developed. They are generally asymptomatic and are included under the benign calcified odontogenic tumors. They are usually discovered on routine radiographic examination.[7]

Levy[5] reported in his study that this hamartoma was caused by separation of partially differentiated epithelial cells from the developing tooth germ.

Von Arx[8] observed the incidence of developmental disturbances of permanent teeth in about 23% of cases after trauma to their predecessors. Any type of trauma to primary teeth may lead to permanent tooth malformation. The extent of malformation depended on the developmental stage of permanent tooth germ on the force of impact and the type of trauma to primary tooth. Most frequent malformation was enamel hyperplasia including enamel discoloration and or enamel defects, crown dilaceration, root malformation includes root dilacerations or to an arrest of root formation, delayed tooth eruption, impaction, root duplication and lateral or vestibular root angulation. Odontome like malformation was observed due to heavy trauma to the predecessor especially after intrusive or total luxation of primary tooth. He considered that odontome like disturbances of permanent human teeth may develop especially after heavy intrusive trauma.

Hitchin[9] reported three cases with a history of trauma to the deciduous dentition at various periods of time which resulted in the formation of odontomes, two cases appeared to occur due to detachment of parts of the epithelial sheath of Hertwig and the term radicular odontome was given. One case in which a portion of enamel organ with already formed enamel proceeded to denticle formation was described as coronal odontome. Though the element of proof of mechanism, whereby trauma may have led to the formation of these odontomes may be lacking, he considered them as traumatic.
odontomes. He suggested that odontome is either inherited or the result of mutant gene or possible interference during tooth development under genetic control. Odontome formation in the present study may be the result of heavy trauma.

Complete disintegration of odontogenic apparatus and sequestration explains the contributory role of trauma for partial anodontia. Polymorphonuclear neutrophils (PMNS) provide an effective host defense not only against bacterial, fungal infections but they are also important to overcome the effect of trauma. Failure of immunological defensive mechanism in the above case can be the cause for disintegration of odontogenic apparatus. The clinical implication of such a condition is partial anodontia.

Andreason et al.[10] reported sequestration of entire tooth germ as a result of trauma. Though Shafer et al.[11] considered partial anodontia is due to genetic mutation, radiation damage, but trauma can also play a role.

Though hand pressure was used as a form of intrusive trauma in various experimental studies, exact magnitude of force applied to the developing tooth germ was not known. This may be the reason for the variation in the histopathological changes of the different research workers. Observation in our study indicates that necessity to use a known magnitude of force as trauma to analyze its effect on the developing tooth germ in different stages and the use of large sample size of experimental groups for statistical significance.

It concludes that light microscopic study of our experiment showed disturbance in the morphology and structure of right maxillary 1st molar of wistar rats including complex odontome due to mechanical trauma. The diversity of morphological and histopathological changes to intrusive force at different intervals of time implies intricate mechanism involved in tooth development. Growth and development after trauma to the tooth germ in various stages of the tooth development is not only based on the trauma at the time of development but also based on the intensity of trauma and other factors like immunological and genetic makeup are to be considered.

REFERENCES

1. Hulse FS. Habits, habitat and hereditary: A brief history of studies in human plasticity. Am J Phys Anthropol 1981;56:495-501.
2. Andreason JO, Raven JJ. Epidermiology of traumatic dental injuries to primary and permanent teeth in a Danish population sample. Int J Oral Surg 1972;1:235-9.
3. William L, Charles FB, Dorthy FM. Odontogenesis of the rat molar. J Dent Res 1953;32:749-71.
4. Andreason JO, Andreason FM. Classification, epidermiology and etiology. In: Andreason JO, Andreason FM, editors. Textbook and colour atlas of traumatic injuries of the teeth. 3rd ed. Copenhagen: Mundsgard pub; 1994. p. 145-65.
5. Levy BA. Effects of experimental trauma on developing first molar teeth in rats. J Dent Res 1968;47:323-7.
6. Taniguchi K, Odamura K, Hayashi M, Funakoshi T, Motakawa W. The effect of mechanical trauma on the tooth germ of rat molars at various developmental stages. A histopathological study. Endod Dent Traumatol 1999;15:17-25.
7. Shekar S, Rao RS, Gunasheela B, Supriya N. Erupted compound Odontome. J Oral Maxillofac Pathol 2009;13:47-50.
8. Von Arx T. Developmental disturbances of permanent tooth following trauma to the primary dentition. Aust Dent J 1993;38:1-10.
9. Hitchin AD. The aetiology of the calcified composite odontomes. Br Dent J 1971;18:260-65.
10. Andreason JO, Sundstroem B, Raven JJ. The effect of traumatic injuries to the primary teeth on their permanent successors. A clinical and histological study of 117 injured permanent teeth. Scand J Dent Res 1971;79:219-83.
11. William G, Shafer, Maynard K, Hine, Barnet M, Levy. Disturbances of development and growth. In: Rajendran R, Sivapathasundaram B, editors. Shafer’s textbook of oral pathology. 6th ed. Elsevier publishers; 2009. p. 45-6

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