Original Article

MORPHOLOGICAL CHANGES IN DENTAL PULP WITH DIFFERENT DEPTHS OF TOOTH PREPARATION

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Abstract

Objective:

This study has been planned to know that under standardized tooth preparation procedure what immediate morphological changes are observed in a different clinical setting, which could be the cause of pulp necrosis later on.

Materials and Methods:

140 intact premolars were used as a subject and were equally divided in four groups (One control and three experimental groups). In each experimental group different depth of tooth preparation procedure was done along with copious irrigation of water and new dental bur for each subject. The teeth were extracted after the procedure and were fixed in 10% formalin solution. Teeth specimens were processed in laboratory and slides were observed under the light microscope.

Results:

In terms of acute inflammatory infiltrate and necrosis 100 % success was observed as none of the study groups revealed this abnormality however other morphological changes including, vacuolated odontoblastic layer with disrupted nuclei, and vasodilatation were observed in all study groups. Chi Square test was applied as inferential statistics.

Conclusion:

Morphological changes do occur in all experimental groups. These changes become more severe and aggressive as the depth of tooth preparation is increased.

INTRODUCTION:

Dental pulp is defined as a soft connective tissue that occupies the central portion of the tooth. This central portion of the tooth is called pulp cavity, which is divided into pulp chamber and root canal. Pulp chamber is that part of the pulp cavity which is present in the crown portion of the tooth and contains coronal pulp. Whereas root canal is that part of pulp cavity which is present in the root portion of the tooth and contains radicular pulp [(1)]. Dental pulp is a sensory organ which is derived from neural crest cells, which proliferate and condense to form dental papilla (the primitive pulp) from which the mature pulp is derived [(2)].

Dental pulp is a firm and resilient connective tissue, principally composed of proteoglycans and glycoprotiens, reinforced throughout by irregularly arranged and interlaced collagen

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fibers. The resilient ground substance of the pulp which is basically a gelatin–like material, limits the intra pulpal pressure to the site of irritation and is not transmitted the whole pulp space. So this configuration of pulpal matrix acts as a barrier against the spread of micro-organisms and toxic products [3].

Microscopically dental pulp has two compartments: odontogenic zone and pulp proper. Odontogenic zone includes odontoblasts (dentin forming cells), the cell free zone, the cell rich zone and parietal plexus of nerves. Pulp proper includes the majority of the pulp and consists primarily of fibroblasts, extra cellular matrix, blood vessels and nerves. In coronal pulp the odontoblasts are columnar in shape whereas in radicular pulp, the odontoblasts are cuboidal or exhibit a flattened morphology [4].

Primary role of pulp is the induction, formation and nutrition of the developing tooth, later it becomes protective and reparative in nature. Low compliance environment, high incidence of sensory nerve innervation and rich microcirculatory components make the dental pulp a unique tissue [3]. The pulp's specialized cells, the odontoblasts and perhaps undifferentiated mesenchymal cells retain the ability to form dentin throughout life. This enables the healthy pulp to partially compensate for the loss of enamel or dentin caused by dental caries or tooth wear, thus maintaining a hard tissue barrier that isolates irritants from the remaining pulp tissue. In response to operative procedure, these odontoblasts may form reactionary dentin or reparative dentin depending upon the extent of injury [5].

Mechanical irritations generated during tooth preparations (cutting a cavity in enamel/dentin or reducing the bulk of tooth for crown) are the most common cause of pulp inflammation [6] . For the purpose of this tooth preparation procedure, a dental drill or handpiece is used in dentistry which is available in a range of speed. According to international standard organization (ISO) a conventional high speed handpiece of speed up to 400,000 rpm (Revolutions per minute) at 2.2 (bar) air pressure, with standard grit diamond bur, used for tooth preparation along with water cooling is considered safe for pulpal health [7].

The earliest signs of pulp reaction to pulpal insults such as tooth preparation are morphological changes which include an overall reduction in the number and size of odntoblast cell bodies, disruption in the odontoblastic cell layer, presence of inflammatory cells and vasodilatation or hemorrhage in cases of severe trauma is visible under light microscope with routine Haematoxylin-Eosin stain. An electron microscopic study on the ultra structural changes of ischemic pulp induced by extraction has shown distinct changes, such as clumping of chromatin, irregular nuclear membrane and swollen mitochondria appear in odontoblasts as early as one hour after extraction [8] It can be speculated that that the lack of oxygen due to circulatory disturbance may be the contributing factor. However no data is available regarding immediate morphological changes in dental pulp after tooth preparation [9].

Heat-energy is considered to be the most injurious event to dental pulp during tooth preparation hence pulp repair can be negatively influenced by the absence of coolant during tooth cutting and bur speed [10].

Cavity or crown preparation, which is of concern as a major cause of pulpal reactions, involves cutting of enamel, dentin and often cementum. The exposed fluid filled tubules permit minute fluid shifts across dentin whenever dentin is exposed to tactile, thermal, osmotic or evaporative stimuli which in turn activate mechanoreceptors and initiate an inflammatory response in the pulp [11]. Dentin hypersensitivity following tooth preparation is a frequent problem in dentistry. Varying degree of odontoblast injury and slight to moderate inflammation in the pulp has been observed. Such pulpal injuries lead to the release of number of inflammatory mediators that may have direct or indirect effects via modulation of trigeminal sensory nerve fibers, on pulpal vasculature. Vasodilatation and increased blood flow are the two major actions seen in the initial phase of pulpal inflammation [12]

Crowns are prepared for those vital teeth, not restorable with simple filling or build up of the lost tooth structure. However it is very difficult to anticipate post crowning health of the pulp, which is not an infrequent occurrence in routine practice. Research has shown that higher incidence (13.3%) of pulp necrosis has been observed in case of full veneer crown preparations as compared to partial veneer preparation in which incidence of pulpal necrosis is lower (5.1%) [13] [14] [15]. A full veneer artificial crown is a type of dental restoration which completely caps or encircles a natural tooth, which has been reduced in thickness of 1.5-2 mm maximum so as to accommodate the bulk of metal and dental porcelain [16].

So the pulp reactivity to tooth preparation can be categorically described as, structural changes, vascular reactions and inflammatory reactions even in the absence of bacteria. Collectively these changes are called as morphological changes which are visible under the light microscope [9].

Since a lot of work has been done with respect to morphological changes in dental pulp after different operative procedures including crown preparation, however no local data is available that how the pulp responds to three different crown preparation procedures. In full veneer metal crowns required tooth reduction is 0.5-0.7 mm, in porcelain fused to metal crowns (Most common type of crowns made in Pakistan, porcelain covering all the surfaces) required tooth reduction is 1.5-2.0 mm and porcelain jacket crowns, in which maximum tooth reduction of 2.0 mm is done. This study therefore aims to investigate the immediate morphological changes in pulp after tooth preparation with water cooling effect and correlating it with three different depths of tooth reduction, comparing changes between control and study groups [17].

MATERIALS AND METHODS:

This experimental study design was approved by the ethical committee of Posgraduate Medical Institute, Lahore. 140 healthy intact premolars were used as a subject in this study which was obtained from the patients of either sex, belonging to the age group 15-25 years. Initially these patients were taken from the Orthodontic department of de'Montmorency College of Dentistry, where they were advised therapeutic extractions of maxillary and mandibular premolars. After evaluating the inclusion criteria, written consent of the patients was taken. On the basis of planed procedure, patients were equally divided into following groups:

In Group A, simple tooth extraction was done as it was the control group in which no procedure was done on the crown.

In Group B, crown preparation was done to the depth of 1.0 mm and tooth was extracted.

In Group C, crown preparation was done to the depth of 1.5 mm and tooth was extracted.

In Group D, crown preparation was done to the depth of 2.0 mm and tooth was extracted.

TOOTH EXTRACTION PROCEDURE: (COMMON IN ALL GROUPS)

Infiltration anesthesia was used for the maxillary premolars and block anesthesia was given in the procedure for mandibular premolars.

Upper and lower universal forceps were used for the extraction of maxillary and mandibular premolars extractions respectively.

POST EXTRACTION PROTOCOL:

Immediately after tooth extraction, root was examined to make sure that it was not broken and was intact. Then the socket was irrigated with normal saline and digital pressure by right hand was applied to squeeze the extraction socket so that expanded buccal cortical plate was returned back. A soaked sterile gauze piece with saline was placed at the extraction site and patient was asked to gently bite on it. Post extraction instructions were given and patient was discharged.

For group B, C and D the tooth extraction procedure was same except that tooth preparation procedure was done before the extraction and rest of the pre and post extraction protocol was same in these groups. However in group A which was the control group, no crown preparation was done and tooth was simply extracted after the local anesthesia.

SPECIFIC ARMAMENTARIUM FOR TOOTH PREPARATION:

1. Standard grit diamond burs according to ISO nomenclature was used.
2. One bur was used only for single tooth preparation. Every preparation was done with a new bur.
3. Tapered needle edge bur No. 010
4. Depth determining burs No. 026, 036.
5. Tapering fissure burs No. 014, 018.
6. Sharp lead pencil for marking groves.
7. NSK high speed handpiece (up to 400,000 rpm and air pressure of 2.2 bars) with appropriate water spray for cooling was used in each preparation.

Care was taken to apply gentle force and intermittent pressure while drilling with handpiece and copious irrigation of water spray through handpiece was also maintained for cooling effect.

REFERENCE POINT FOR TOOTH PREPARATION:

Crown preparation procedure is carried out keeping in mind the following rules:

1. For axial reduction of teeth the tapering fissure bur was placed parallel to the long axis of the tooth.
2. Tip of the tapering fissure bur was placed at the gingival margin which is the reference point of subsequent tooth reduction.
3. Shoulder preparation was done on all surfaces of premolars.

TOOTH PREPARATION PROCEDURE FOR GROUP B: (CROWN REDUCTION UP TO 1.0 MM)

First of all tapered needle edge bur was used to break inter proximal contacts so that during crown preparation procedure, adjacent teeth were not damaged.

Then depth determining groves were made with depth cutter bur No. 026 on the buccal and palatal/lingual surfaces of crown, which gave the guideline for crown reduction up to 1.0 mm.

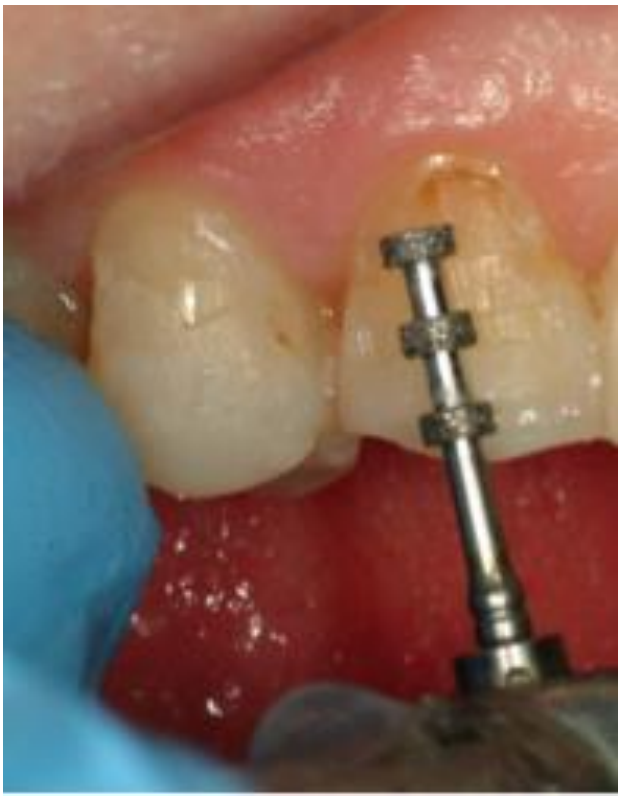

Figure 1.

These groves were marked with lead pencil. Later the tapering fissure bur No. 014 was used for axial reduction of crown. When the depth determining groves were blended with each other and till the lead markings vanished, this confirmed that desired tooth reduction was achieved. Similar steps were carried out for occlusal reduction using same burs.

TOOTH PREPARATION PROCEDURE FOR GROUP C: (CROWN REDUCTION UP TO 1.5 MM)

Inter proximal tooth reduction was done with similar technique as in group B and for same purpose. However depth determining groves were made with depth cutter bur No. 036 on the buccal and palatal/lingual surfaces of crown, which gave the guideline for crown reduction up to 1.5 mm. These groves were also marked with lead pencil. Later the axial and occlusal tooth reduction was done in the similar way as in group B.

TOOTH PREPARATION PROCEDURE FOR GROUP D: (CROWN REDUCTION UP TO 2.0 MM)

Inter proximal tooth reduction was done with similar technique as in group B and C and for same purpose. However depth determining groves were made with tapering fissure bur No. 018 on the buccal and palatal/lingual surfaces of crown, which gave the guideline for crown reduction up to 2.0 mm and were marked with lead pencil. Later the axial and occlucal tooth reduction was done in the similar way as in group B and C.

TISSUE SAMPLING:

Immediately after tooth extraction apical third of the root apex was cut off with the tapering fissure bur No. 014 so that fixation solution could penetrate easily up to coronal pulp and fix it. All extracted premolar specimens were preserved in biopsy jars containing 10% formalin. Separate jar was used for each premolar specimen and labeled with patient's name, age, group number and date.
Each specimen was first decalcified in 5% nitric acid solution in the petri dish and decalcification was confirmed by the dissection needle. Later routine processing was done in the histology laboratory of Postgraduate Medical Institute, Lahore to prepare slides for microscopic examination.

HISTOLOGICAL EXAMINATION:

Slides stained with Hematoxylin and Eosin (H&E) were studied under light microscope with 100x, 200x and 400x magnification to see the histological architecture of dental pulp.

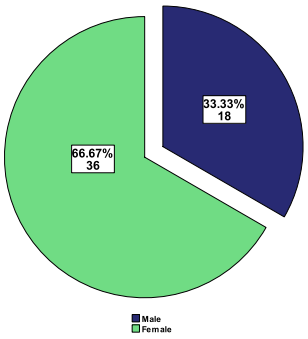

Figure 2: Age Distribution (Years)

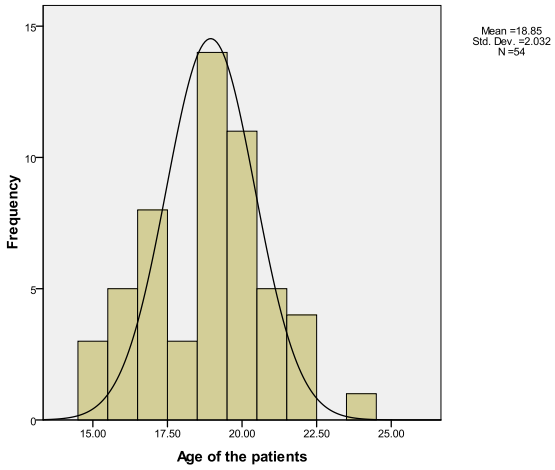

Figure 3: Gender Distribution

Data was analyzed by Pearson Chi-square/Fisher exact test to observe association between morphological changes and groups. P-value ≤ 0.05 was considered as statistically significant.

RESULTS:

Age of patients:

The mean age of 54 patients was 18.85±2.03 years with a minimum of 15 years and maximum of 24 years of age (**Fig. 2)**. A total of 18 (33.33%) males and 36 (66.67%) females were included in the study **(Fig. 3).**

ODONTOBLASTIC ZONE:

Odontoblastic zone was found and recognized among all the specimens of Group A, B and C, while in Group D, it was present in 31 (89%) of total 35 subjects while in 4 (11%) it was not recognized. The difference in presence of Odontoblastic zone was statistically associated with study groups (p-value=0.006).

CELL-FREE ZONE:

Cell-free zone which is present between the odontoblastic zone and cell-rich zone was found and recognized in 100% subjects of all the study groups.

CELL-RICH ZONE:

Cell-rich zone was found and recognized in all the specimens of Group A, B and C, whereas in Group D, it was absent in 4 (11%) subjects only. Chi Square test showed that presence of

cell-rich zone was significantly associated with study groups (p-value=0.006).

COLUMNAR ODONTOBLASTIC LAYER:

The frequency for normal undisrupted layer of columnar Odontoblasts demonstrated varying results. Columnar odontoblastic layer was normal in all subjects of Group A. In Group B, 31 (89%), in Group C 16 (46%) and in Group D 08 (23%) subjects had normal layer. The difference in normality of columnar odontoblastic layer was highly associated with difference of groups (p-value=0.000).

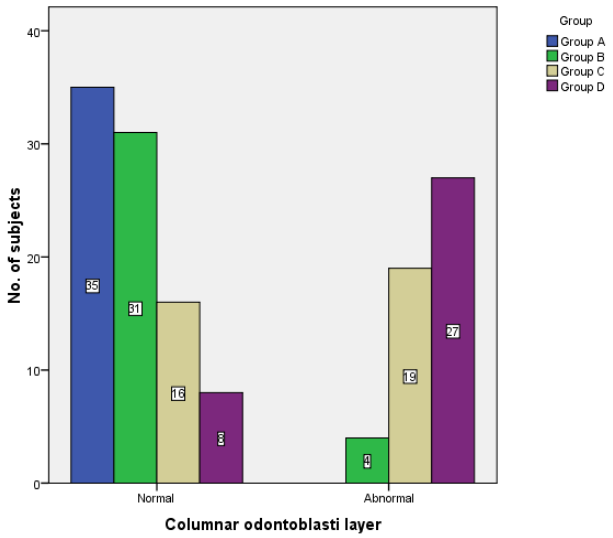

Figure 4: Frequency 0f Columnar Odontoblast Layer In Different

Chi Square: 59.858, **df:** 3, **p-Value**= 0.000

NUCLEI:

The examination for determining the Nuclei to be normal or abnormal showed interestingly different results. All specimens in patients of Group A had normal nuclei, while 20 (57%) specimens in Group B had normal value for nuclei. Contrarily, 26 (74%) specimens in group C and 33 (94%) in Group D gave abnormal values for nuclei (disrupted nuclei), with only 26% and 6% specimens with normal nuclei in these groups respectively. The difference in pattern of nuclei normality was statistically associated with respect to different study groups (p-value=0.000).

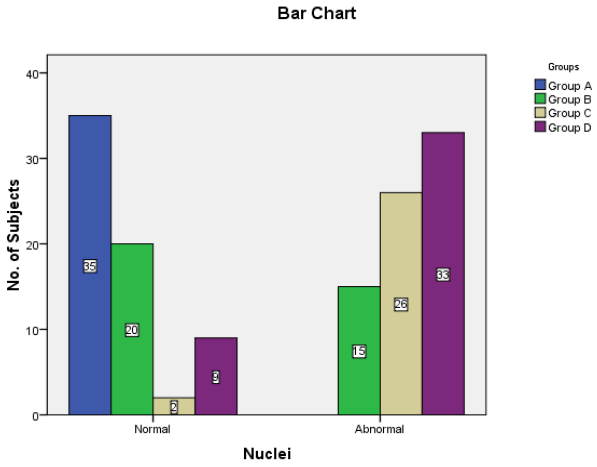

Figure 5: Observations of Nuclei in Study Groups
Chi Square: 31.354, **df:** 3, **p-Value**= 0.000

VASODILATATION:

The examination regarding presence or absence of vasodilatation demonstrated that all specimens in Group A and C did not show any vasodilatations, while it was absent in 34 (97%) specimens of Group B and 24 (69%) specimens of Group D. Again, the difference in pattern of vasodilatation was significantly associated with respect to different study groups (p-value=0.000).

MICRO HEMORRHAGE:

Micro hemorrhage was not found in all specimens of Group A, B & C while in Group D, it was absent in 31 (86%) of the specimens. The difference in presence of Micro hemorrhage was associated with different study groups (p-value=0.06).

ACUTE INFLAMMATORY INFILTRATE:

Acute inflammatory infiltrate was observed to be absent in 100% specimens of all the four groups.

DISCUSSION:

Operative procedures like crown preparation require the use of high speed handpiece which certainly effects the dental pulp ranging from slight to severe morphological changes, under different clinical conditions. However immediate response of dental pulp to these high speed preparations can be classified in

three groups. Structural changes include the displacement of the odontoblastic nuclei into the dentinal tubules being the most important ones. Vascular reactions include vasodilatation and hemorrhage in interstitial spaces. Inflammatory reaction includes presence of acute inflammatory cells in the absence of bacteria.

Previous studies have shown 13.3% incidence of pulpal necrosis associated with full veneer crown preparation as compared to partial veneer crown preparation. Secondly immediate changes in dental pulp also vary with different types of preparations depending upon remaining dentin thickness. Current study has been conducted to evaluate morphological changes in dental pulp with different depths of tooth preparation so that we could know the immediate effects of these iatrogenic procedures which can be the consequences of clinical symptoms later on. In control group normal morphologic pattern of dental pulp has been observed in all the slides showing all four zones and their respective cells which are clearly identifiable. The odontoblastic layer appeared uninterrupted and continuous with columnar shape cells. The central pulp revealed loose connective tissue with dispersed fibroblasts and isolated few collagen fibers as the dental pulp was young. Frequency of different parameters in the study groups revealed that in group B and C odontoblastic zone was present in all subjects being studied and was clearly identifiable. Frequency of cell-free zone is significantly present in all study groups which shows normal morphologic feature of dental pulp. Columnar odontoblastic layer like palisade appearance is typical normal feature of dental pulp which is disturbed by the presence of vacuolization in this layer in response to any iatrogenic tooth preparation documented by different studies in the past and an important determinant of morphologic change in dental pulp. In the current study this vacuolization in the odontoblastic zone has been observed in all study groups and it like other parameter this significant change also increases in its frequency as the depth of tooth preparation is increased. It is quite obvious from the results that this change has been observed in 54% of subjects of group C (1.5 mm tooth reduction) whereas in group D where almost two third of the subjects showed this change (77%) (Fig 6).

Presence of undisrupted nuclei in the odontoblastic cell bodies is a feature of normal dental pulp. In group B where 1 mm tooth reduction was done, disruption of nuclei in odontoblastic layer appeared in 15 subjects out of 35 (Fig 5), which significantly increases to almost double (33 subjects) in number in group D where 2 mm tooth reduction was done, representing effects of tooth preparation as the depth is increased leading to increased frequency of this morphologic change as a response of dental pulp to this procedure. Similar morphologic changes of vacuolated odontoblastic layer and disrupted odontoblastic nuclei have been observed in group B with 11% and 43% respectively.

Frequency of vasodilatation has been observed 31% at the maximum in group D (Fig 6) and only 1 subject of group B showed this change, however in group D, 11 subjects out of 35 significantly showed this change which is obviously due to greater depth of tooth preparation leading to more frictional trauma to underlying dental pulp.

A study conducted in Japan in 2007 regarding effects of orthodontic tooth movements on the morphological and hemodynamic effects on pulp also showed that degenerative changes like, vacuolization of odontoblast layer, circulatory disturbances and transient reduction in pulpal blood flow all are reversible once the active orthodontic force was removed, however in humans it is still not clear whether the orthodontic forces have similar biologic effects on dental pulp or its recovery [18] [19]

Occurrence of interstitial hemorrhage has been noted in only 4 subjects of group D showing an aggressive morphologic change as a result of tooth preparation up to 2 mm (Fig 7). However it is also important to note that, in those subjects who showed this change, it was always associated with other morphologic changes as well like, vasodilatation, disrupted nuclei in the odontoblasts and vacuolization in odontoblastic zone which shows that as the depth of tooth preparation is increased that chances of severe morphological changes also increases accordingly.

Results of the similar study conducted in Romania showed that dentin preparation resulted in inflammatory cells in dental pulp in the absence of bacteria and also that this degree of inflammation corresponded with different depths of tooth preparation [(9)].Another study being conducted in 2001 on human dental pulp to check the response of different restorative materials in deep cavities revealed that moderate inflammatory response and disorganization of pulp tissue are associated with remaining dentin thickness [(20)]. Here it is worth mentioning that dental pulp in none of the study groups showed any signs of inflammation or presence of any acute inflammatory infiltrate. That means crown preparation procedure even at 2 mm depth is within safe limit and it does not develops any reversible or irreversible pulpitis. Since in the current study the observations were confined to the immediate changes in dental pulp, this might be possible that this acute inflammatory infiltrate could be a positive finding after 24 hours, which are very unlikely to develop immediately after crown preparation.

An important significant factor being calculated in the study groups is the total time taken for the tooth preparation to complete. Because the time duration taken to prepare the tooth using high speed hand piece is an important determining factor of extent of thermal and frictional heat damage even in the presence of copious irrigation of water. Intermittent tooth preparation for longer duration is considered less iatrogenic as compared to continuous tooth preparation even for shorter duration. However while working clinically on patients it is not possible neither feasible to continuously keep on using high speed hand piece, so keeping that thing in mind an average time taken for tooth preparation has been recorded for each subject and its correlated with morphological changes observed afterwards. In all study groups minimum tooth preparation time on an average has been 22 minutes while its maximum average value has been 41 minutes. However it is interesting to note that there is only one subject in group B which showed the morphologic changes like vacuolization in columnar odontoblastic layer, disruption of odontoblastic nuclei and vasodilatation, while time taken for tooth preparation for this subject has been noted as 40 minutes. Similarly micro hemorrhage observed in 4 subjects of group D and tooth preparation time recorded for these subjects also falls in maximum range, which confirms its negative influence over the tooth preparation procedure.

In previous studies the minimum age group of patients included is 9 to 12 years and mandibular or maxillary premolar has been taken as a subject. Normal eruption timings for all human premolars are 10 to 12 years on an average and time for their root completion is around 13 to 15 years respectively. So the studies in which they have included patients of 9 to 12 years of age, how can they justify their results since a partially erupted premolar and the one with incomplete root formation or an open apex cannot be considered as ideal tooth for the procedure of crown preparation specially when we want to see the immediate morphological changes in their pulp. But in the current study 15 years age has been taken as minimum age limit by that time apices of all premolars are complete. Later it has also been confirmed by periapical X-rays about the root completion and apex closure in that specific premolar. In this way the possibility of false positive results has been eliminated and this study truly describes the morphological pattern in dental pulp in response to different depths of tooth preparation.

CONCLUSION:

These results reveal that tooth preparation procedure is not completely a traumatic for dental pulp even under standardized conditions and some morphological changes do occur in all experimental groups. These changes become more severe and aggressive as the depth of tooth preparation is increased. However we can only minimize the consequence of pulp degeneration and necrosis if we manage to reduce the time of tooth preparation and we make sure that for every tooth preparation, a new bur is used with copious irrigation of water.

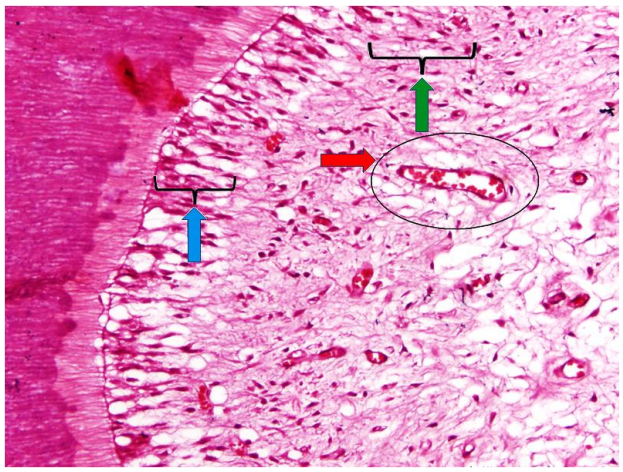

Figure 6: Photomicrograph of dental pulp of group (C) showing changes in morphological pattern. Vacuolated odontoblastic zone with disrupted nuclei (blue arrow), cell free zone dispersed with cell rich zone (green arrow), vasodilatation with hemorrhage (red arrow). H & E stain X 200.

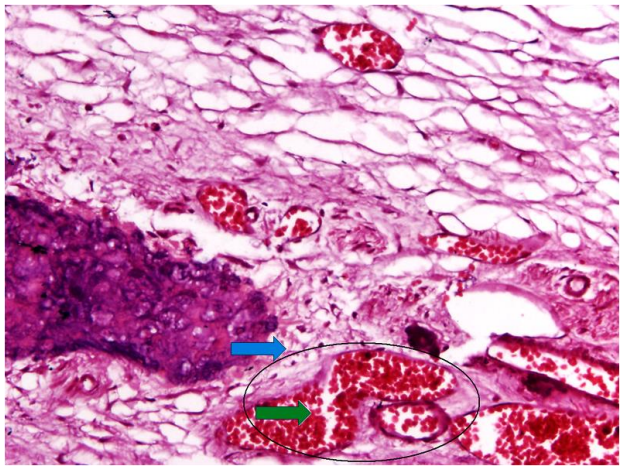

Figure 7: Photomicrograph of dental pulp of group (D) showing changes in morphological pattern. Vasodilatation (blue arrow) and micro hemorrhage (green arrow). H & E stain X 200.

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A WISE MAN FIRST THINKS AND THEN SPEAKS AND A FOOL SPEAKS FIRST AND THEN THINKS.

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