INTRODUCTION

Endodontically maxillary premolars are difficult to treat because of variability in their morphology. The various factors including number of roots, canal configuration, the direction and longitudinal depressions of the roots, and various pulp cavity configuration all play a key role in treatment outcomes of dental procedures on these teeth. These procedures range from apexectomy, root canal treatment on internal structure and placement of prosthetics appliances and aesthetics on external morphology of premolars. Studies have identified certain external as well as internal morphological features of the human teeth. These features as identified by Vertucci and later by Sert have helped us differentiate between the anterior (incisors and canine) and posterior (premolars and molars) sets of teeth. Further, some of these characteristics have been identified as unique for the various populations of the world in terms of ethnicity, age and gender. For example, North American, Far Eastern and Turkish populations have identified significant racial variations in root and canal anatomy of maxillary pre-molars.

The techniques used to identify the root canal morphology vary from radiology to in vitro techniques. Demineralization and staining is one such in-vitro technique that keeps instrumentation away from the root canal and prevents disruption of its natural anatomy. In addition this technique allows for a three dimensional picture of root canal by stereomicroscopy and further classification of root canal system. Though many of classifications are in vogue, a blend of Vertucci and additional classification given by Sert provides a much more holistic overview to the internal arrangement of these pre-molar canals.

The study was designed to classify premolar morphology using a standardized procedure for our population to help dental surgeons treating these patients in planning their procedures and reduce complications. The main aim was to compare first and second premolars canal morphology and its classification.
ethnicity and their gender was recorded on the collection sheets. Teeth with resorbed roots, fillings and peri-apical cysts were excluded from the study.

After collection teeth were washed, cleaned and stored in distilled water containing thymol crystals (antifungal agent). The external gross anatomical parameters of tooth/root length were recorded in the pre-processing phase using vernier caliper. Relative root length was then calculated as $RRL = \frac{ARL}{TL} \times 100$ ($RRL$, relative root length; $ARL$, absolute root length; $TL =$ Tooth length). The number of roots as well as presence of mesial root depression was also noted. The teeth were then processed to observe internal anatomical parameters by fixing in 10% formalin solution for 24 hours and shaken after every 8 hours. They were then treated with 5% solution of sodium hypochlorite (Dejung, Korea) for next 48 hours for removal of organic tissues. An access cavity was made with high speed rotary hand-piece (NSK-PANA max) and straight fissure stainless steel burr with moderate water spray during cutting. The pulp floor was removed. With small size endodontic file #15 and most obvious canals were cleaned of any loosely necrotic pulpal tissues present, if any. Teeth were then decalcified with 5% nitric acid and then dehydrated with series of 70%, 80%, 90% and 100% concentrated ethanol (AnalaR 100%). Hematoxylin dye (Merck liquid) was injected with 23 gauge needle under high vacuumed compression suction negative pressure ensuring efficient flow of dye into the canals. Each tooth was dipped in liquid methyl salicylate for 72 hours to make it transparent.

Transparent teeth were examined under stereomicroscope, (Olympus Corporation Tokyo-Japan, Modal SZ61); Photographs were taken by camera fixed on lens of stereomicroscope with resolution power, 2560 x 920 megapixels. Stereoscopic anatomy of the root canal configuration was classified according to Vertucci and Sert as well as lateral canals and canal isthmi were observed. The classification was validated by triangulation from two other expert researchers. Statistical analysis was done by using Statistical Package for Social Science (SPSS version 20.0) for both qualitative and quantitative per adult patient was included in the study of ages between 20 and 60 years. Patients were probed for linguistic ethnicity and their gender was recorded on the collection sheets. Teeth with resorbed roots, fillings and peri-apical parameters of tooth/root length were recorded in the pre-processing phase using vernier caliper. Relative root length was then calculated as $RRL = \frac{ARL}{TL} \times 100$ ($RRL$, relative root length; $ARL$, absolute root length; $TL =$ Tooth length). The number of roots as well as presence of mesial root depression was also noted. The teeth were then processed to observe internal anatomical parameters by fixing in 10% formalin solution for 24 hours and shaken after every 8 hours. They were then treated with 5% solution of sodium hypochlorite (Dejung, Korea) for next 48 hours for removal of organic tissues. An access cavity was made with high speed rotary hand-piece (NSK-PANA max) and straight fissure stainless steel burr with moderate water spray during cutting. The pulp floor was removed. With small size endodontic file #15 and most obvious canals were cleaned of any loosely necrotic pulpal tissues present, if any. Teeth were then decalcified with 5% nitric acid and then dehydrated with series of 70%, 80%, 90% and 100% concentrated ethanol (AnalaR 100%). Hematoxylin dye (Merck liquid) was injected with 23 gauge needle under high vacuumed compression suction negative pressure ensuring efficient flow of dye into the canals. Each tooth was dipped in liquid methyl salicylate for 72 hours to make it transparent.

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2. ANOVA (one way) & post hoc Tukey: Means were compared for significance in quantitative variables with more than two sub groups within a group.

3. Independent sample T test: T test was applied to see measure of association between the quantitative variables of the two groups.

A 95% confidence interval was considered significant, $P \leq .05$.

**RESULTS**

The outcome measures of this experimental study were root lengths, root forms and mesial depression on gross observation and canal configuration, exit and isthmi and lateral canals on microscopy.

The age of the patients ranged from 20-60 years with mean age of $40.33 \pm 11.48$ years and $41.29 \pm 10.39$ years for first and second premolar groups, respectively while ratio of males to females was equal. Linguistic ethnic groups Pashtun and Hindkun were in a ratio of 2:1.

The average tooth length ranged from 19-26 mm ($21.39 \pm 1.31$ mm) and 17.5-25mm ($21.13 \pm 1.39$ mm) for first and second premolar groups, respectively. The average root lengths ranged from $-16$ to $-15$mm with approximately 1mm difference in lengths in both groups ($n=210$). The differences in root length were only significant for buccal roots (Figure 1). The relative root length was calculated for each group as a percentage of total tooth length ranging from $-68\%$ for palatal roots to $71\%$ for buccal (double roots) and $-74\%$ for single roots. Each of these roots had either an apical or lateral canal exit with the majority of the single or double rooted teeth having an apical canal exit ($-94\%$, $83\%$, respectively). On the other hand only $4\%$ and $32\%$ teeth had lateral exit in first and second premolars respectively ($P<.001$, Chi-square test, Figure 2).

As illustrated in Figure 3 root forms were classified as: single rooted, two separate roots, laminated free at apex1/3, laminated free at middle1/3 and full fused bifid root. In first premolars, five different types of external root forms were observed while four forms were observed in second premolars. The single root predominates in second premolar teeth by a far greater margin; 4:1 as compared to 2:1 in first premolars ($P<.001$, chi-square test, Figure 4). However, there was no significant difference between males and females or ethnicity in distribution pattern of root forms.

Comparison between first and second premolars revealed highly significant
difference for parameter “mesial surface depression” (P<.001, chi-square test) with 76.2% first premolars with mesial surface depression. In both groups teeth with various forms of two roots had a significantly higher percentage of this depression as compared to single rooted teeth (P<.001, one way Anova & post hoc Tukey test).

Further details of canal configuration and presence of canal isthmi and lateral canals were observed under stereomicroscope. Based on modified root canal configuration (Vertucci & Sert), twelve types of canal configurations were found in sample teeth as illustrated in (Figure 5). In the first premolars, ten types of canal forms were identified of which the greatest percentage were type IV (2 canal, 2 exit) (61.9%), while in second premolars seven types were identified with type II (35.2%) as the predominant morphology (P<.001, Chi-square test, Figure 6).

Furthermore there was a positive correlation between the external root morphology and the canal configurations (0.196, Pearson correlation) with significance of p=0.004. Thus, in all single and double rooted morphologies Type IV canal configuration was the commonest finding in first premolars. On the other hand in the second premolars Type II along with Type IV was the commonest configuration in all root morphologies. There was no significant difference of canal variations between males and females (p=0.893, Fisher’s exact) or between pashtun and hindkun groups (p=0.587, Fisher's exact). Though for both demographics Type IV was the commonest form in first premolars whereas second premolars had a more variable picture of Type I, II, IV and VI. Our population based on gender and ethnicity does not appear to favour Types VIII, IX, X, XI, XII, XIII, XIV, XV and XVI types.

Approximately, 80% of the first premolars and 90% of second premolars had no identifiable lateral canals. Half of the lateral canals were observed in apical third of roots in both first and second premolar.

Position and presence of inter canal communication was observed with no inter canal communications in ~92% of the premolars. Comparatively high percentage of inter canal isthmi was found in second premolar as compared to first premolar in middle and apical thirds of the root (P<.05, Chi-square test).

DISCUSSION
Ethnic differences in teeth root anatomy have long been recognized, but the frequency of these variations in root form and canal anatomy has not been documented.14 This study aimed to identify the gross anatomical and microscopic anatomic variability of root and canal configuration of maxillary premolars in our population in general and to compare the anatomical difference of maxillary first premolars to maxillary second premolars. Various methods are used to identify these variations whereas our main emphasis was on the clearing technique, as it is as effective as other expensive radiographic methods like CBCT in vitro.15,16

In our study there was equal distribution of premolars in both genders but with majority belonging to the Pashtun linguistic group. Based on modified root form classification we identified ten types of canal forms in our population with Type IV being the predominant morphology in first premolars and Type II in the second premolars. We also identified five main root forms; single root, two separate roots, laminated root free at apex 1/3, laminated root free at middle 1/3 and full fused bifid
premolars; on the contrary significantly greater percentage of the first premolars had mesial surface depressions.

Tooth lengths in different ethnic groups of the world e.g. Denmark, Sweden, U.S.A and Turkey have shown no significant differences between first and second premolars as shown in our study. However Kim et al. in his study showed that Asians had shorter tooth length than Caucasians in a study conducted in Korea. Though in our study the absolute root lengths in both premolar groups were more or less similar but as demonstrated in studies by Pecora, Black, and Anderson the buccal root length was relatively longer in the second premolars. Similarly Tsukiyama et al., working on Asian and White Caucasian populations identified a similar trend. The understanding of possible variations in the root lengths is very important for a dentist to aid him/her in devising a more precise treatment plan for the patients. These lengths are calculated on in vitro samples whereas a dentist works on in vivo teeth with most of the root embedded into the gum. Therefore in addition we postulated a relative root length as a better indicator for dentists to estimate the root length by observation of visible crown which would be in the ratio of 1:3 (crown: root) in double roots and 1:2 in single root teeth.

Historically studies have documented root forms as single or double root form but we further elaborated sub varieties of the two rooted teeth forms depending on extent of lamination. The need for this further sub classification arose because of a lack of uniformity in accepted guidelines on assigning root forms. Especially in bifurcated teeth with free apical third; as the question was whether to classify them as single rooted or double rooted. Such teeth were considered double rooted in studies conducted by Walker, Awawdeh, Nelson and Pineda. We followed the same criteria and placed such teeth in the double rooted category; labeled as laminated but separate at apical third. Though there was an overall preponderance of single root teeth; almost all sub types of double rooted teeth were also identifiable in our population similar to the results by Vertucci, Kertal, et al., AJ Chaparro, et al., Atieh, et al. and Awawdeh, et al. The first premolars of Chinese and Mongoloid origin are mostly single rooted contrary to our findings of the second premolar with this feature. Muana, et al. in Jordanian roots in our population. However, there was no significant difference based on gender or linguistic ethnicity. In addition we also observed other identifiable parameters such as relative root length, lateral canal exits, mesial depression, inter-canal communication and external root formations. The second premolars had a significantly greater frequency of lateral canal exits as compared to first premolars; on the contrary significantly greater percentage of the first premolars had mesial surface depressions.
population, Pecora et al. in Brazilian population and Trope, et al. in Negroid patients also found that single rooted teeth were comparatively more in second premolars than double rooted teeth. Though we could not identify a significant difference in the two premolar groups based on the linguistic ethnicity (pashtun versus hindkun) in this study. The variations reported by these studies in various ethnic groups may indicate a potential clue to identification of premolars in the larger context of races: Caucasian, Asian, South American etc. Similar to a study conducted on a Spanish population we could not identify any significant dimorphism in the premolar teeth.

Looking into further detail on the internal canal configurations the variability in results; as reported by various studies becomes even more complicated. Our study reports Type IV and Type II as the commonest forms in first and second premolars, respectively as reported by Al-Ghananeem et al. and some studies on Turkish and Jordanian populations. On the other hand Type III was the commonest reported form by another study on Turkish population as well as one on Saudi population. Thus even within countries there appear to be regional differences. We may surmise that as Turkey, Jordan and Saudi Arabia are in same continent “Asia” so their populations show nearly same pattern of canal configuration i.e. Type II, III and IV. These differences may to some extent be explained by the various techniques used; a 2D image from a radiograph would have its own limitations including quality of the resolution and missing information, on the other hand a 3D image of the actual canal by clearing and dye injection produces clearer visualization of the canals in all dimensions. It well worthwhile to compare these techniques especially with the advent of cone-beam computed tomography techniques for in-vivo use.

The majority of premolars in our study had two canals as the commonest configurations are; Types II, IV and VI as also shown by Bellizzi, et al, Caliskan, et al. However a Brazilian study and Vertucci as well identified Types I and III with a one canal configuration. Thus it is imperative for the endodontist to understand this morphological character which is irrespective of an external root form because missing an extra canal may lead to treatment failure. Further complicating matters for the dentist who works mainly blindly to occlude or treat all canals and their communications is the presence of lateral canals and inter-canal communications (isthmi) along the length of the root. Mostly both of these were found at apical third of the root in our study and also by Kertal, et al. However, Vertucci found more lateral canals at middle third of the roots while canal isthmi were more at apical third of the roots in both first and second premolars.

Our study showed that there was a mesial surface depression, starting approximately, at the cemento-enamel junction to middle of the root with more than two thirds of the first premolars with this distinguishing feature as compared to the second premolars as did Marlin, et al. (USA) and Pecora, et al. (Brazil). Thus dentists should take particular care while placing posts in such teeth to avoid perforation in such roots.

Vertucci found more lateral exits and less central exits in first premolars as well as in second premolar. This finding is opposite to our finding where both first and second premolar teeth with one root form had apical root canal exit while two root forms showed apical as well as lateral exit. One reason maybe aging or excessive occlusal forces on the teeth which leads to thickening of apical cementum resulting into displacement of apical foramen further from center. This aspect maybe further explored as Vertucci did not identify any demographics in his study.

CONCLUSION

We identified a range of variations in the canal configurations in first and second premolars. The first premolars have mostly two roots with a type IV canal configuration (2 canals and 2 exits), while second premolars have single roots on external morphology but with a greater variation internally of: type I, II, IV and type VI canal form. Thus, second premolars have a much more diverse configuration ranging with mostly two canals and a variety of exits. Thus for a successful endodontic procedure a sound anatomical knowledge of the canals would lead to better treatment and less chances of failure.

Though we could not identify any gender of linguistically identifiable features in our population this study adds to the larger body of international studies conducted on various populations the world over and may form the basis for further forensic identification studies.

ACKNOWLEDGEMENT

We are thankful to Khyber Medical University for partial funding of this project to AS. We would also like to acknowledge technical services of Mr. Jameel, Dental technician DHQ, Kohat and Dr Nadia Aaman Associate Professor Fauji Foundation for her expert opinion.

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