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ARAŞTIRMA / RESEARCH

Influence of establishing a glide path on the amount of apically extruded debris and the preservation of root canal anatomy

Kayma yolu oluşturulmasının apikalden çıkan debris miktarına ve kök kanal anatomisinin korunmasına etkisi

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Abstract

Purpose: This study aimed to investigate the effect of glide path preparation before shaping with reciprocating single-file, rotary single-file and multiple-file systems on the debris extrusion from the apical, and on the root canal transportation.
Materials and Methods: One hundred twenty curved mesial root canals of mandibular first molars were randomly distributed to six groups (n=20). The root canals were prepared with Reciproc in Group R, OneShape in Group OS, and ProTaper Next in Group PN. In the last three groups (RG, OSG and PNG), a glide path was created before the shaping instruments used in the first 3 groups. The pre- and post-preparation weight were measured by using a 10-5 microbalance. Pre- and post-preparation of the root canals were scanned by using cone-beam computed tomography (CBCT).
Results: The debris extrusion in RG and OSG was significantly higher than R and OS, respectively. However, no significant differences were found between PN and PNG. Considering the root canal transportation, RG showed lower than R for 5 mm and 7 mm levels and the OSG group showed lower than OS for all levels. Creating a glide path significantly increased the shaping time for OS and PN groups.
Conclusion: In advance of shaping with a single-file system in curved canals, creating a glide path preserves canal anatomy. However, it may lead to increase apical debris extrusion.
Keywords:. CBCT, debris extrusion, glide path, reciprocation, root canal, single-file.

Öz

Amaç: Bu çalışmada, resiprokal tek eğe, tam rotasyon tek eğe ve çok basamaklı eğe sistemleriyle şekillendirme öncesi kayma yolu hazırlığının apikalden debris çıkışına ve kök kanalının yer değiştirmesine üzerindeki etkisinin araştırılması amaçlanmıştır.
Gereç ve Yöntem: Mandibular birinci molar dişlerin yüz yirmi eğimli meziyal kök kanalı rastgele altı gruba dağıtıldı (n=20). Kök kanalları Grup R'de Reciproc, Grup OS'de OneShape ve Grup PN'de ProTaper Next ile hazırlandı. Son üç grupta (RG, OSG ve PNG), ilk 3 grupta kullanılan şekillendirme aletlerinden önce bir kayma yolu oluşturuldu. Preparasyon öncesi ve sonrası ağırlık 10-5 mikro terazi kullanılarak ölçülmüştür. Kök kanallarının preparasyonu öncesi ve sonrası konik ışınlı bilgisayarlı tomografi (KIBT) kullanılarak tarandı.
Bulgular: RG ve OSG'deki debris ekstrüzyonu, sırasıyla R ve OS gruplarından önemli ölçüde daha yüksekti. Ancak, PN ve PNG arasında anlamlı fark bulunmadı. Kök kanal yer değiştirmesine bakıldığında, RG, 5 mm ve 7 mm seviyeleri için R grubundan daha düşüktü ve OSG grubu tüm seviyeler için OS'den daha düşüktü. Bir kayma yolu oluşturmak, OS ve PN grupları için şekillendirme süresini önemli ölçüde arttırmıştır.
Sonuç: Eğimli kanallarda tek eğe sistemi ile şekillendirme öncesinde bir kayma yolu oluşturmak kanal anatomisini korur. Bununla birlikte, apikal debris ekstrüzyonunun artmasına neden olabilir.

Anahtar kelimeler: KIBT, debris ekstrüzyonu, kayma yolu, resiprokal, kök kanalı, tek eğe.

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INTRODUCTION

Root canal shaping procedures are challenging for the narrow and curved root canals[1-3]. It has been reported that complications such as transportation, step formation, loss of working length and perforation may be encountered during the root canal preparation[4,5]. The introduction of nickel-titanium (NiTi) file systems reduced these failures[6,7]. The number of files in a shaping system was reduced owing to the increased durability of NiTi files. However, the taper of the files was increased to obtain a sufficient shaping[8,9]. Berutti et al. stated the requirement of creating an initial glide path, especially in narrow and curved root canals[10]. Endodontic glide path was described as a flat round tunnel extending from the canal orifice to the end of the physiological apical foramen[11]. Creating a glide path in the root canal contributes to developing resistance against the file breakage while it preserves the original anatomical shape of the root canal[12]. One of the glide path files, ProGlider (Dentsply Maillefer, Ballaigues, Switzerland) is a novel rotary glide path file made of M-wire alloy. ProGlider has a tip thickness of 0.16 mm, an advancing taper from 2% to 8% and a square cross-section with four incisive sides. The other glide path file, OneG (MicroMega, Besançon, France) was made of M-wire alloy which has a tip thickness of 0.14 mm, a taper of 3% and an asymmetrical cross-section with three incisive sides.

Debris composed of irrigants, bacteria, dentin remnants and necrotic tissue can spread to the periradicular tissues and cause periapical inflammation and post-treatment pain during the instrumentation[13]. Limited studies indicated that creating a glide path by using a rotary glide path file system reduced the apical debris extrusion before the single-file shaping systems[14,15].

Single-file systems have two different concepts which are reciprocating and continuously rotating motion. Reciproc and One Shape represent these single-file systems with different instrument designs and metallurgy. OneShape (MicroMega, Besancon, France) is used in full continuous rotation, while Reciproc (VDW, Munich, Germany) is used in a reciprocating motion. The ProTaper Next (Dentsply, Maillefer, Ballaigues, Switzerland) is a continuous rotary system manufactured from M-wire. According to the manufacturer, most of the canals can be prepared by using only the first 2 files among the 5 files of the ProTaper Next system. Although studies are suggesting that the use of glide path files before shaping with single-file systems[10,16,17], there are also studies reporting that there was no effect[7,18]. Moreover, the effectiveness of glide path files was controversial for multiple-file systems[19,20].

There was available knowledge on the ability of single-file systems to preserve the root canal anatomy and on the quantity of debris extruded by the files. However, there were few studies on the contribution of a creating glide path in advance of shaping with a single-file system. The purpose of this study was to explore the effect of glide path on reciprocating single-file, rotary single-file and multiple-file systems in curved root canals in terms of root canal transportation, apical debris extrusion and preparation time. The null hypothesis tested was that no significant differences would be between the groups with and without glide path on debris extrusion, root canal transportation and consuming the time for root canal preparation.

MATERIALS AND METHODS

Sample

The present study was found appropriate according to the report of Çukurova University Faculty of Medicine Clinical Research Ethics Committee with a number of 2015/41-1. The first molar teeth with excessive crown destruction and being given an indication of extraction obtained from patients who applied to Çukurova University Faculty of Dentistry Endodontic Clinic were surgically removed and included in this study in line with the informed consent of the patients. The collected teeth were disinfected. Experiments were carried out by a senior researcher and 2 assistant professors in Çukurova University Faculty of Dentistry Research Laboratory.

The sample size was calculated with G*Power 3.1 software (Heinrich Heine University, Dusseldorf, Germany) with an alpha-type error of 0.05 and 95% power, taking into account previous studies[12]; this revealed that at least 15 teeth per group were required for transportation analysis and 18 teeth were required for debris analysis. To strengthen the statistical analysis during the experiment, 20 teeth per group were used. Mesial canals of one hundred and twenty extracted human mandibular first molar teeth with Vertucci class VI were used in this study as a result of ethical consent from the patients[21]. The mesial canals having completed apical formation were selected according to 25°-35° of curvature of the root canal by using the Schneider method[22]. The root canals were examined with ISO #8 K-file. The working length was determined as 12 mm, which was 1mm shorter than the canal terminus under ×25 magnification by using an operating microscope (Zeiss Opmi Pico, Jena, Germany). The teeth were placed into the Eppendorf tubes (Sigma Aldrich, Steinheim, Germany) to collect debris and the tubes were placed into vials covered with aluminum folio.

Root canal preparation

The mesial root canals were randomly distributed to six groups (n=20). In the first 3 groups, the glide path was not created. In the R group, the canals were prepared by using the Reciproc R25 file (25 mm, 8%) with pecking motion at the "Reciproc ALL" program. In the OS group, the canals were prepared using OneShape file (25 mm, 6%) with in-and-out movements without pressure at speed of 350 rpm and 2,5 N/cm torque. In the PN group, the canals were prepared by using ProTaper Next X1 (17 mm, 4%) and X2 (25 mm, 6%) with in-and-out movements at speed of 300 rpm and 2,5 N/cm torque. In the other three groups, the glide path was created before the preparation of the root canals. In RG and PNG groups, the glide path was created with ProGlider. In the OSG group, the glide path was created with One G. Following every three in-and-out movements, the flutes of the file were cleaned up during both creating the glide path and using the shaping systems. Each file was only used to prepare two root canals on the same sample. VDW silver endodontic motor (VDW, Munich, Germany) was employed in all groups.

Each root canal was irrigated with a total of 5 ml distilled water by using a side-vented manual irrigation needle (30-gauge, DiaDent, Almere, Netherlands) with an in-and-out motion after every 3 pecking movements. The needle tip was placed 2 mm shorter than the working length at the last irrigation session. All root canal preparation procedures were carried out by one skilled specialist.

Debris collection

The experimental setup was prepared on the basis of the study of Myers and Mon Montgomery[23]. Eppendorf tubes were measured with 10^{-5} sensitive scales (Radwag, Radom, Poland) 3 times and the average value was recorded as the empty tube weight before the preparation. The root canals were placed into 1.5 ml Eppendorf tubes with silicone rubber covers and were sealed with a gingival barrier. In order to balance the air pressure inside the Eppendorf tube and the outside air pressure, a needle of 27 gauge was put in the tube. The Eppendorf tubes were placed in aluminum-wrapped vials and immobilized. After the shaping procedure was completed, Eppendorf tubes were removed from the prepared setup. The debris accumulated on the root surface was washed with 1 ml of distilled water. The Eppendorf tubes were kept in an incubator (Memmert, Schwabach, Nürnberg, Germany) at 70°C for 5 days in order to evaporate the liquid. The weight of Eppendorf tubes was measured three times again and average values were taken. The amount of debris extrusion was calculated by getting the weight of the first and the second measurements.

Root canal transportation analysis

Pre- and post-preparation, CBCT images (Planmeca Promax 3D Mid, Helsinki, Finland) were captured at the set of 90kV, 6.3mA, 15 second exposure time and a slice thickness of 75mm. The images obtained were analyzed by using the OsiriX program (Pixmeo SARL, Bernex, Switzerland). The method developed by Gambill et al. was used in order to determine root canal transportation[24]. The shortest distance from the outer wall of the tooth to the outer wall of the root canal was measured on the pre- and post- preparation images for each determined level which were 2 mm, 3 mm, 5 mm, and 7 mm from the minor apex. Root canal transportation was calculated by using the formula following: $|\,(x_1 - x_2) - (y_1 - y_2)\,|$, where "$x$" indicates the length in the mesial direction, "y" indicates the length in the distal direction. Pre-preparation is presented with the "$_1$" symbol and post-preparation is presented with the "$_2$" symbol. The distance calculations on all CBCT images were analyzed separately by two skilled researchers and the results were validated with consensus in the presence of a third experienced clinician.

Preparation time measurement

A stopwatch was used to calculate the working time, which was included the preparation phase, irrigation, cleaning of the flutes of the files and checking of the working length. With the end of the last irrigation, the counter was stopped. This time record was repeated in all experimental groups.

Statistical analysis

Statistical analyzes were performed by using the SPSS program for Mac version 26 (IBM SPSS Inc, Chicago, IL) The distribution of the obtained data from the experiments of root canal transportation, debris extrusion, and calculation of preparation time was analyzed by applying the Shapiro-Wilk test. Multiple comparisons of the non-parametric distributed data belonging to all experiments were analyzed with the Kruskal Wallis test with the Bonferroni correction. Pairwise comparisons of the data were performed by the Mann-Whitney U test. The data was evaluated with 95% of confidence interval (p=.05).

RESULTS

Descriptive statistics and pairwise comparisons of the groups were represented in Table 1. All groups produced debris from the apical of the root canal. Creating a glide path before shaping systems resulted in a greater amount of debris extrusion from the apex of the root canal compared without a glide path. The quantity of debris was significantly increased in RG and OSG groups compared to R and OS groups (p=.000 and .01, respectively). However, no statistically significant difference was detected between PN and PNG groups (p =.09). When all groups were compared, the most debris extrusion was found in the RG group, and the differences were found to be significant comparing R and OS groups (p=.000) The amount of debris extrusion of the groups was displayed with Box plot in Figure 1.

Pairwise and inter-level comparisons of root canal transportation values were shown in Table 2. Root canal transportation was observed in all groups. The glide path preparation significantly reduced the root canal transportation for rotary single-file system (OS - OSG groups) at all levels. There was a statistically significant decrease at 5- and 7-mm levels for the reciprocating single-file system (R - RG). No significant differences were found for the multiple-file system (p>.05).

Table 1. Debris extrusion values (mg) from apical of root canal and pairwise comparison of groups.

Groups	Mean	SD	Minimum	Maximum
R	0.34^{A}	0.17	0.13	0.97
RG	0.69^{B}	0.32	0.24	1.27
p	0.000			
OS	0.33^{A}	0.09	0.18	0.52
OSG	0.46^{B}	0.18	0.22	0.80
p	0.014			
PN	0.41^{A}	0.13	0.21	0.61
PNG	0.52^{A}	0.24	0.20	0.93
p	0.099			

Different superscript letters in the same column indicate significant differences between groups. Statistically significant at $p < 0.05$. SD: Standard deviation, R: Reciproc, OS: OneShape, PN: ProTaper Next, "G" suffix: Glide path.

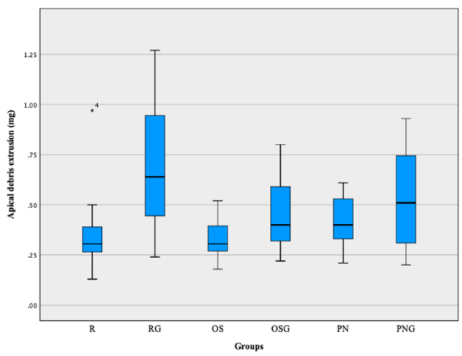

Figure 1. Box plots of apically extruded debris values of groups

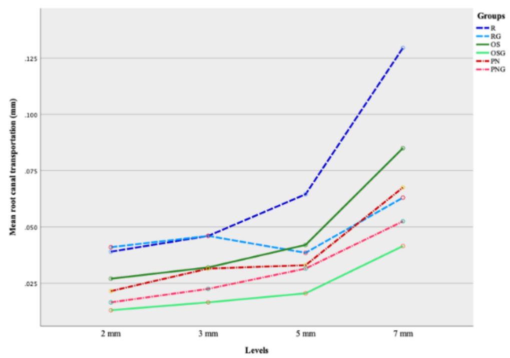

Figure 2. Graph of mean root canal transportation values of groups according to 2 mm, 3 mm, 5 mm, 7 mm levels

Table 2. Root canal transportation values (mm) and statistical analysis of the groups

	2 mm		3 mm		5 mm		7 mm		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	***p***
R	0.039^{A,a}	0.024	0.046^{A,a}	0.03	0.064^{A,a}	0.029	0.129^{A,b}	0.051	.000
RG	0.041^{A,a}	0.023	0.046^{A,a}	0.03	0.038^{B,a}	0.022	0.063^{B,a}	0.050	.495
p	.795		.880		.003		.000		
OS	0.027^{A,a}	0.016	0.032^{A,a}	0.012	0.042^{A,a}	0.032	0.085^{A,b}	0.036	.000
OSG	0.013^{B,a}	0.009	0.016^{B,a}	0.009	0.020^{B,a}	0.016	0.041^{B,b}	0.028	.000
p	.002	.000	.000		.011		.000		
PN	0.021^{A,a}	0.013	0.031^{A,a}	0.024	0.033^{A,a}	0.020	0.067^{A,b}	0.036	.000
PNG	0.016^{A,a}	0.011	0.022^{A,a}	0.016	0.031^{A,ab}	0.021	0.052^{A,b}	0.028	.000
p	.263		.362		.846		.178		

Capital superscript letters show the differences between groups in the columns, small superscript letters show the differences between levels in the rows. Statistically significant at $p < 0.05$. SD: Standard deviation, R: Reciproc, OS: OneShape, PN: ProTaper Next, "G" suffix: Glide path.

Table 3. The total time values (s) required for shaping the groups

Groups	Mean	SD	Minimum	Maximum
R	248.65^{B}	58.31	169	405
RG	293.35^{AB}	86.79	152	491
OS	157.25^{C}	33.36	108	247
OSG	276.95^{B}	66.86	189	455
PN	233.60^{B}	51.10	153	363
PNG	351.25^{A}	85.48	230	551
p	.000			

Different superscript letters in the column indicate significant differences between groups. Statistically significant at $p < 0.05$. SD: Standard deviation, R: Reciproc, OS: OneShape, PN: ProTaper Next, "G" suffix: Glide path.

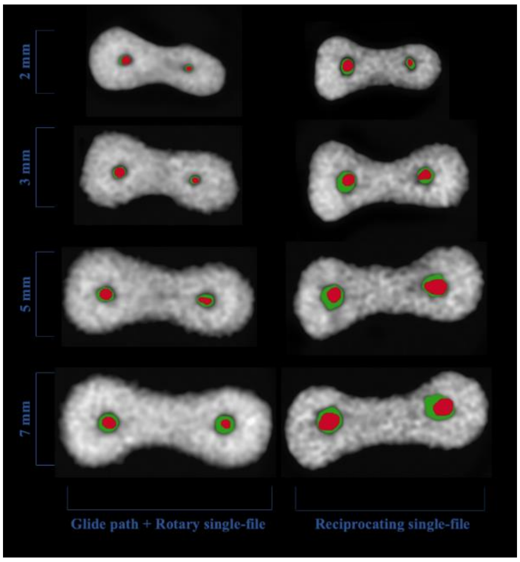

Figure 3. Pre- (red) and post-preparation (green) superimposed CBCT images of root canal transportation were represented at the 2 mm, 3 mm, 5 mm and 7mm levels by selecting one sample from each of the OSG groups with the lowest value and the R group with the highest value.

There was a significant difference among the levels in all groups except the OS group (*p*=.000). Significantly higher root canal transportation occurred at 7 mm level compared to the 2, 3 and 5mm levels for R, PN, RG groups (*p*<.05). Similarly, there was a significant difference at 7 mm level compared to 2- and 3-mm levels for OSG and PNG groups (*p*<.05). Mean root canal transportation values (mm) at four different levels were exhibited in Figure 2.

Significant differences were found between the groups at all levels when the Kruskal Wallis test was employed (*p*=.000). At the level of 2mm, the most root canal transportation occurred in R and RG groups and both of them were significantly higher than OSG and PNG groups (*p*<.05). At the level of 3 mm, canal transportation was significantly lower for the OSG group (*p*<.05) than the others, except PN and PNG groups. At the level of 5 mm, the root canal transportation was significantly higher for the R group than OSG, PN and PNG groups (*p*<.05). At the level of 7 mm, the highest root canal transportation was occurred in the R group compared to others, except the OS group (*p*<.05). The superimposed CBCT images selected among the highest and the lowest values of root canal transportations were represented in Figure 3.

The mean root canal preparation time of the groups was given in Table 3. Glide path preparation significantly increased the shaping time for OS and PN groups (*p*<.05). However, no significant difference was found for the R group (*p*>.05). Compared to the other groups, the rotary single-file system was completed root canal preparation with the shortest duration (*p*<.05).

DISCUSSION

The effect of creating a glide path on 3 different NiTi shaping systems was investigated in terms of debris extrusion and root canal transportation in this study. Creating a glide path before reciprocating single-file and rotary single-file systems increased the quantity of extruded debris and decreased the amount of root canal transportation. Thus, the null hypothesis was rejected. There were limited studies evaluating the effect of creating a glide path on the shaping system. Debris extrusion of three different single-file systems were evaluated with and without creating a glide path and it was reported that debris extrusion was decreased in the groups with glide path[15]. Reciprocating and rotary single-file systems were compared with and without glide path and no difference was found in terms of debris extrusion[14]. In this study, creating a glide path before shaping with single-file systems was significantly increased the debris extrusion. However, the glide path did not have an effect on the debris extrusion of the multiple-file system. The difference between single- and multiple-file systems might be explained by cutting a significant amount of dentin in a short time with a single instrument, similar to that Bucheli et al. showed in their systematic review and meta-analysis[25]. Furthermore, creating a glide path before the shaping system may allow the apical third of the root canal to clear the residues and to expand a smooth round tunnel to the apex[11]. The increased debris extrusion in the glide path groups may be explained by the great quantity of debris produced by single-file shaping systems and by pushing the debris into the space enlarged in the apical-third. In a study evaluating the effect of different kinematic movements on the amount of remaining debris in the root canal with micro-computed tomography (micro-CT), the continuous motion of the rotary files induced upward removal of debris along with the flutes of the file, while each backward motion of the reciprocating files compacted the debris along walls of the dentin and pushed them beyond the apex[26]. Similar to the above study, cutting more dentin from the root canals caused by reciprocating motion may explain the difference between OSG and RG groups.

Although R and OS groups showed similar debris values, a significant difference occurred when a glide path was created before these shaping systems. This result may be related to the lack of an escape pathway in the apical direction when a glide path was not created.

In a study comparing the six shaping systems, the OneShape system caused significantly greater debris extrusion compared to ProTaper Next and Reciproc systems[27]. Bürklein et al. investigated Reciproc, OneShape, F360 and Mtwo systems, and the OneShape system caused significantly less debris extrusion compared to the Reciproc system[28]. In another study, a significant difference was not found between the systems comparing Reciproc, OneShape and ProTaper and the least debris extrusion occurred in the OneShape system[29]. In our study, no significant difference was found between the R, OS and PN groups.

There are several studies emphasizing the importance of creating a glide path for the preservation of root canal anatomy. Zanette et al. showed that creating a glide path would provide a guide path along the entire root canal and would reduce tight contact between canal wall and shaping instrument[30]. Patino et al. studied the relationship between creating a glide path and the fracture of instruments[31]. It was reported that rotating glide path instruments reduced the rate of fracture and occurred less root canal transportation. Zheng et al. reported reduced canal transportation in their research comparing PathFiles and K-files, the ProGlider file combined with a single-file system[16]. In this study, the use of the glide path system before the shaping system decreased the root canal transportation at almost all levels and in all groups.

Reciprocating single-file systems were reported to be able to reach working length without creating a glide path[12,32]. Carvalho et al. evaluated the Reciproc system in terms of root canal transposition at 2- and 3-mm levels with different glide path systems and found no significant difference[18]. In our study, whether or not to create a glide path before shaping with the Reciproc system caused similar root canal transportation at 2- and 3-mm levels, while the differences between 5- and 7-mm levels were significant. The fact that the glide path reduced root canal transportation for the reciprocating single-file may be attributed to the severity of curvature at these levels.

Creating a glide path before the OneShape system significantly reduced root canal transportation at all levels. Few studies evaluated rotary single-file systems in terms of root canal transportation[33,34]. Furthermore, no reference was found about the effect of creating a glide path with OneG before shaping with OneShape. Using PathFile prior to OneShape in simulated S-shaped canals did not show any difference[7]. Comparing different glide path systems with different shaping systems, Vyver et al. used ProGlider and OneG systems before the OneShape system. There was no significant difference at all levels in terms of root canal transportation[17].

In this study, creating a glide path before the multiple-file system did not result in a significant reduction in terms of root canal transportation. In a study of Elnaghy and Elsaka, the use of ProGlider before the ProTaper Next system significantly decreased the root canal transportation compared to the ProTaper Next system alone[19]. In another study, no significant difference was found to evaluate the ProTaper Next system with different glide path systems[20].

According to the results of comparing all groups, higher root canal transportation occurred in the R group than the others at all levels. The reason for this result may be shown due to having a taper of .08 of Reciproc in the first 3 mm. The cross-section shape of the file was another effective factor in the occurrence of root canal transportation[12]. In another study that evaluated NiTi instruments with different kinematic characteristics[35], Reciproc showed the highest value of transportation because of its S-shaped wide cross-sectional features.

In a recent study comparing ProTaper Next, OneShape and EdgeFile, which were systems produced with different heat treatment procedures, no significant difference was found between the groups in terms of root canal transportation[36]. Similarly, in our study, the transportation value of the OneShape group was higher than the ProTaper Next group. Hayashi et al. reported that the bending properties of the files with triangular cross-sections were more successful than those with rectangular cross-section[37]. However, in this study, the PN group showed less root canal transportation with no significant difference compared to the OS group. This may be attributed to the number of files used.

The glide path files were selected according to the manufacturer's recommendations and the glide path was prepared with OneG and ProGlider. These files were produced by M-wire heat treatment and had similar physical properties. Vyver et al. recently compared transportation of K-files, ProGlider and One-G files in curved root canals; the two engine-driven glide path groups performed similar apical canal transportation values[38]. Güneş and Yeter found no difference in terms of debris extrusion caused by the OneG file and ProGlider file in their study comparing 5 different glide path files[39]. In the study of Ha et al. comparing 3 different glide path files, they reported that the OneG file produced more debris than the ProGlider[13]. The glide path files were not compared in this study. Although more debris extrusion occurred with OneG in studies comparing glide path systems, in our study, the OSG group caused the least debris extrusion among the glide path groups. Based on this result, it may be concluded that the shaping system was more important than the selection of the glide path system in terms of debris extrusion.

The mean root canal transportation values of the groups were compared at levels of 2 mm, 3 mm, 5 mm and 7 mm from the apical foramen in the present study, due to the possibility of occurring a mishap was found more at these levels[39]. There was a significant difference in all groups except the RG group, and a dramatic increase was observed at 7mm in all groups.

There are some limitations of this study. The disability to imitate the pressure of periapical tissues created an inability to reflect clinical use for debris extrusion. The other limitation was that micro-CT could provide more detail than CBCT. However, there was a strong correlation between the two methods[40] and CBCT was more economical and faster than micro-CT. Another limitation was that the main shaping systems included different production processes. The shaping systems used in this study were produced with different techniques. ProTaper Next and Reciproc are manufactured of a thermally processed NiTi alloy, while OneShape is manufactured of conventional NiTi. The fact that natural teeth have different variations is another factor affecting the standardization of the study.

Within the limitations of this study, the following could be deduced that creating a glide path before rotary and reciprocal single-file systems helped to preserve the original anatomy of root canals with severe curvature. However, it should be noted that creating a glide path before root canal shaping with single-file systems might increase debris extrusion from the apical. Furthermore, creating a glide path prolonged the preparation duration of the curved root canals.

Yazar Katkıları: Çalışma konsepti/Tasarımı: CK, OY, BE; Veri toplama: CK, BE; Veri analizi ve yorumlama: CK; Yazı taslağı: CK; İçeriğin eleştirel incelenmesi: CK, AD; Son onay ve sorumluluk: CK, OY, BE, ŞY, AD; Teknik ve malzeme desteği: OY; Süpervizyon: CK; Fon sağlama (mevcut ise): yok.
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Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.
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Author Contributions: Concept/Design : CK, OY, BE; Data acquisition: CK, BE; Data analysis and interpretation: CK; Drafting manuscript: CK; Critical revision of manuscript: CK, AD; Final approval and accountability: CK, OY, BE, ŞY, AD; Technical or material support: OY; Supervision: CK; Securing funding (if available): n/a.
Ethical Approval: This in-vitro study was found suitable according to the report of Çukurova University Faculty of Medicine Clinical Research Ethics Committee with number of 2015/41-1.
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