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RESEARCH ARTICLE

QUALITATIVE AND QUANTITATIVE ANALYSIS OF APICAL MICROLEAKAGE OF DIFFERENT ENDODONTIC SEALERS.

Vallari Jain[1], Prateeksha Chowdhry[1], Mamta Kaushik[2], Roshni Roshni[3] And Neha Mehra[3].

1. Master of Dental Surgery (M.D.S), Department of Conservative Dentistry and Endodontics, Army College of Dental Sciences, Secunderabad, India.
2. Professor and Head, Department of Conservative Dentistry and Endodontics, Army College of Dental Sciences, Secunderabad, India.
3. Reader, Department of Conservative Dentistry and Endodontics, Army College of Dental Sciences, Secunderabad, India.

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Abstract

Purpose: The purpose of the study was to compare the apical microleakage of five root canal sealers using qualitative dye penetration method and quantitative dye extraction method.

Materials & Methods: Fifty single rooted premolars were prepared with ProTaper Universal Rotary File System up to size F3 and obturated with lateral condensation technique using F3 master cone and accessory cones. They were divided into five groups (n=10) according to the sealer used for obturation. Group 1-ZOE based sealer, Group2-Calcium Hydroxide based sealer, Group 3 –Epoxy resin-based sealer, Group 4- Silicon based sealer and Group 5- MTA based sealer. The apical microleakage was determined using two different techniques: Dye penetration and Dye extraction. Statistical analysis was performed using Kruskal-Wallis test and Post hoc Mann Whitney U test [IBM SPSS for Windows, Version 19.0 (SPSS; Chicago, IL, USA)]

Results: The difference in apical microleakage was statistically significant according to dye penetration method (Kruskal-Wallis, $p<0.001$, Post Hoc Mann Whitney U test, $p<0.05$) between the following groups: A & D, B& C, B & D, C & E, D& E. No significance was seen according to dye extraction technique.

Conclusion: MTA and Calcium Hydroxide based sealers displayed least apical leakage while ZOE based sealer displayed highest apical leakage. The results seen with both techniques were more or less similar.

Introduction:-

The key to successful endodontic therapy is obtaining a good three-dimensional seal to prevent infection and reinfection [1]. The root canal sealers play a vital role in achieving this by sealing the voids, accessory canals and apical ramifications, with the possibility of attaining good sealing ability to dentin, less solubility, good biocompatibility, mineralization to dentin and formation of calcified tissues to seal the apex[2-5]. A variety of sealers

Corresponding Author: -Prateeksha Chowdhry
Address: - Master of Dental Surgery (M.D.S), Department of Conservative Dentistry and EndodonticsArmy College of Dental Sciences, Secunderabad, India.

have been tested for this purpose ranging from the conventional zinc oxide eugenol sealers to the latest resin, MTA and Calcium hydroxide-based sealers[4,5].
The sealing ability of root canal sealers has been extensively tested in the past using various methods such as dye penetration[6-8], bacterial leakage[9], electrochemical technique[10,11], fluid filtration[12,13], glucose leakage test[14], radioisotope penetration[15], and capillary flow porometry[16]. However, the results from different studies have been contradictory due to the diversity of traces and criteria used in various leakage studies.

Dye penetration technique has been the most commonly used technique as it is simple. But the drawback of this technique is that it is a qualitative test and yields a high level of variation[17].

The dye extraction technique involves spectrophotometric analysis of the volume of tracer. It has gained popularity due to its quantitative nature[17,18].

The aim of this study was to compare the apical microleakage of five different root canal sealers using qualitative dye penetration method and quantitative dye extraction method.

Methods: -

Fifty human single rooted premolars extracted for orthodontic purpose were collected from the Department of Oral and Maxillofacial Surgery, Army College of Dental Sciences and stored in 0.1% thymol after manual cleaning, scaling to remove soft tissue debris followed by autoclaving at 121℃, 15 lbs. pressure for 15 minutes. The teeth were selected based on the following criteria: no gross coronal or root caries, absence of resorption or fracture; presence of mature, fully formed apices; single canal (confirmed from the radiograph) with canal diameter no greater than size 30.The teeth were then decoronated below the cementoenamel junction using a diamond disk under water coolant, to obtain roots with a standard length of 16 mm. Access cavities were prepared with a small round Endo access FG 1 bur, (Dentsply, Tulsa Dental Specialties, United States) in a high speed handpiece, and patency of the canal was established with a #10K-file (Mani.Inc, Japan). Cleaning and shaping was done using crown down technique with ProTaper Universal Rotary files till size F3 (Dentsply, Tulsa Dental Specialties United States). A size 15 K-file (Mani.inc) was used between each ProTaper instrument to verify the canal patency followed by irrigation with 2 ml of 2.5% Sodium Hypochlorite (Vishal Dentocare, Pvt Ltd, Ahmedabad, Gujarat) delivered with a 27-gauge needle (5ml disposable syringe, Dispovan, India). After completing the preparation, the canal received a final rinse of 10 ml 17% EDTA (Dentwash, Prime Dental, Bhiwandi, Then, India) (pH = 7.7), followed by 10 ml of 2.5% sodium hypochlorite (Vishal Dentocare, Pvt Ltd, Ahmedabad, Gujarat) and the canals were dried with paper points (Protaper Universal Absorbent points, Dentsply, Tulsa Dental Specialties, United States).

The teeth were randomly divided into five groups of ten samples each according to the sealers used for obturation: Group-A: Epoxy Resin based sealer (AH Plus, Dentsply, Konstanz, Germany), Group B- MTA based sealer (MTA Fillapex, Angelus, Brazil), Group C- Silicone based sealer (Gutta Flow2®, Coltene/ Whaledent, Germany), Group D- ZOE based sealer (Pulp Canal Sealer, EWT, Sybron Endo, West Collins Avenue Orange, CA), Group E- Ca $(OH)_2$based sealer (Sealapex, Kerr, Orange, California, United States).

The sealer was applied with a lentulospiral and obturation was performed using F3 master cone (Dentsply, Tulsa Dental Specialties, United States) followed by lateral condensation using accessory cones. The coronal access cavities of all roots were sealed with IRM (Dentsply, Tulsa Dental Specialties, United States). The obturated samples were stored for 24 hours at 37^0C and 100% humidity in an incubator to allow setting of the sealer.

In this study, a sample of root canal treated tooth with a sealer free obturation was used as a positive control and another sample of tooth in which no root canal treatment was performed was selected as a negative control.

Apical seal was evaluated for all the samples using the two methods i.e. dye penetration and dye extraction.

Dye Penetration Method:
All the samples (n=50) were covered with two layers of nail varnish except at the apical 3mm. The apices of the teeth were dipped in glass vials for 24 hours at 37^0C in a neutral buffered 2% Methylene Blue solution, under normal atmospheric pressure. After removal from dye, the teeth were rinsed under tap water for thirty minutes and the varnish was removed with polishing disks mounted on a handpiece. The teeth were allowed to dry before sectioning to prevent spreading of dye.

The teeth were then carefully sectioned longitudinally using a diamond disc to expose the gutta-percha–sealer interface at its greatest diameter throughout the length of the root canal.

The extent of the dye penetration was measured in millimeters (Table-I) with a calibrated eyepiece mounted on a Stereomicroscope (Olympus, Tokyo, Japan) at 16 X magnification. [Figure-1 [A-E]

Dye Extraction Method:
The same two root halves were then subjected to quantitative dye extraction. Each tooth sample was stored in 2 ml of concentrated (65 wt. %) nitric acid for 3 days [Figure-2[A]] and centrifuged at 14,000 rpm for 5 min. 2 ml of the supernatant layer from each sample [Figure-2[B]] was transferred to plastic cuvettes (Quartz Cuvette, standard 10mm, 3.5 mL, spectrometer cell). Samples were read by an automatic spectrophotometer (Beckman, DU 520) at 550 nm using concentrated nitric acid as the blank.

Results: -

The results obtained in dye penetration technique showed highest microleakage by ZOE based sealer (Group D), followed by Silicone based (Group C), Resin based (Group A), Calcium hydroxide based (Group E) and least by MTA based sealer (Group B). [Table- I]

Table I:-Mean values± Standard deviation [SD] of depth of dye penetration.

Groups	Dye penetration [in mm]				
	N	Minimum	Maximum	Mean	SD
Positive Control				1.25	
Negative Control				0.10	
A] A H Plus	10	.25	1.25	.78	.362
B] MTA Fillapex	10	.10	1.00	.46	.293
C] Gutta Flow 2	10	.25	1.50	1.02	.432
D] Pulp Canal Sealer [EWT]	10	.25	2.25	1.50	.624
E] Sealapex	10	.10	1.00	.53	.305

The results were statistically significant according to Kruskal-Wallis test at p value <0.001. Post hoc Mann Whitney U test demonstrated significant differences at p value < 0.05 between the following groups: A & D, B& C, B & D, C & E, D& E [Table-II].

Table II:-Mean difference and p value [dye penetration]

Groups		Mean Difference	P value
A] A H Plus	MTA Fillapex	0.32	0.052
	Gutta Flow 2	-0.24	0.190
	Pulp Canal Sealer [EWT]	-0.72	0.007*
	Sealapex	0.25	0.143
B] MTA Fillapex	Gutta Flow 2	-0.56	0.005*
	Pulp Canal Sealer [EWT]	-1.04	0.001*
	Sealapex	-0.07	0.631
C] Gutta Flow 2	Pulp Canal Sealer [EWT]	-0.48	0.063
	Sealapex	0.49	0.015*
D] Pulp Canal Sealer [EWT]	Sealapex	0.97	.002*

*- statistically significant [p<0.05]

The results of the spectrophotometer indicate the light absorption of the Methylene Blue in the resin-dentin interface which shows the microleakage of the restoration [Table-III].

Table III:-Mean values± Standard deviation [SD] values using dye extraction technique

Groups	Dye extraction [absorbance]				
	N	Minimum	Maximum	Mean	SD
Positive Control				-2.8	
Negative Control				-0.1	
A] A H Plus	10	-3.30	-.70	-1.55	.809
B] MTA Fillapex	10	-3.10	-.60	-1.56	.717
C] Gutta Flow 2	10	-2.40	-.10	-1.64	.755
D] Pulp Canal Sealer [EWT]	10	-3.80	-.90	-1.90	1.071
E] Sealapex	10	-2.10	-.60	-1.46	.591

However, the differences within the groups were not statistically significant, due to which, the results obtained with the two techniques could not be compared statistically.

Discussion: -

Root canal microleakage is a complex phenomenon which is influenced by various factors such as root filling materials, techniques, physical and chemical properties of sealers and presence of smear layer etc[19]. Successful obturation requires the use of materials and techniques capable of densely filling the root canal system and providing a fluid tight seal to prevent reinfection[3]. Different endodontic filling materials and techniques have been introduced in the past to improve the apical seal thereby preventing microleakage[20].

Various methods have been advocated for testing microleakage in teeth. Tooth immersion in various types of dyes such as eosin, methylene blue, black India ink, Procion brilliant blue, etc. was first reported by Grossman in 1939[21]. It one of the most widely used since it involves a simple technique and equipment[18].

Methylene Blue has a very small molecular size, with a low molecular weight, lower than that of bacterial toxins, is inexpensive, easy to manipulate, has a high degree of staining and it allows easy quantitative measurement of the extent of dye penetration by linear measurement technique. Moreover, it diffuses easily and is not absorbed by the dentin matrix apatite[21-23].

Despite the popularity, dye penetration studies have several limitations. The random longitudinal dentinal sectioning employed, may have a high probability of the section not being made through the deepest dye penetration point. The fluid movement may be hindered by the air entrapped in voids along the root canal filling[8]. So, the dye extraction method was also used in this study since it involves recovering all of the dye that penetrates the apex, thereby overcoming the limitations of the dye penetration technique[18].

The evaluation was done using a stereomicroscope as it gives a three-dimensional view of the surface to be examined and does not require any pre-treatment of the specimen[23].

In the present study, the core filling material and the obturation techniques were kept constant and smear layer removal was ensured prior to obturation in all the samples for standardization. So, the probable factor affecting the microleakage could be the variable properties of the sealers used.

Results stated that MTA Fillapex and Sealapex showed least amount of leakage as compared to the other sealers according to the dye penetration method whereas Sealapex, AH plus and MTA Fillapex showed least amount of microleakage according to the dye extraction method.

The improved sealing ability of MTA Fillapex is attributed to the setting mechanism of MTA which results in hydration of anhydrous mineral oxide compounds to produce calcium silicate hydrate and calcium hydroxide phases, which causes expansion thereby enhancing the seal and minimizing leakage. MTA fillapex shows an optimized flow due to presence of nanoparticles helping it to penetrate and fill lateral canals[24].

Sealapex which is a Calcium Hydroxide based sealer had significant amount of volumetric expansion during setting because of water absorption[25].

AH plus is known for its good physico-chemical properties such as its good dimensional stability, better penetrability, low solubility and self-adhesiveness. These factors contribute to its satisfactory apical sealing ability[26].

Camps and Pashley suggested that there was no correlation between dye penetration, the fluid filtration and dye extraction techniques. Both, fluid filtration and dye extraction techniques give similar results as they consider the porosity of the interface between the filling material and the root. They are both based on a quantitative measurement of the liquids passing through these interfaces. The dye extraction technique, however, may be better than the fluid filtration technique as the filtration values tend to diminish with time, because the water penetrates all the irregularities until a plateau is reached[21]. The dye penetration technique despite being most common, simple and easy cannot be completely reliable because it is based on randomly sectioning the root into two pieces which may not necessarily be the level of the deepest dye penetration[18].

Conclusion: -

1. MTA Fillapex and Sealapex provide better apical seal against microleakage as compared to other sealers. The results seen with both the techniques were more or less similar.
2. The dye extraction technique is more accurate, moreover, is based on the quantitative measurements of the dye passing through voids or porosities between the canal filling material and the root walls. Hence, it may be considered as a promising technique for microleakage evaluation.
3. Further studies using other methods of microleakage detection, may provide more scope for comparison and reliability.

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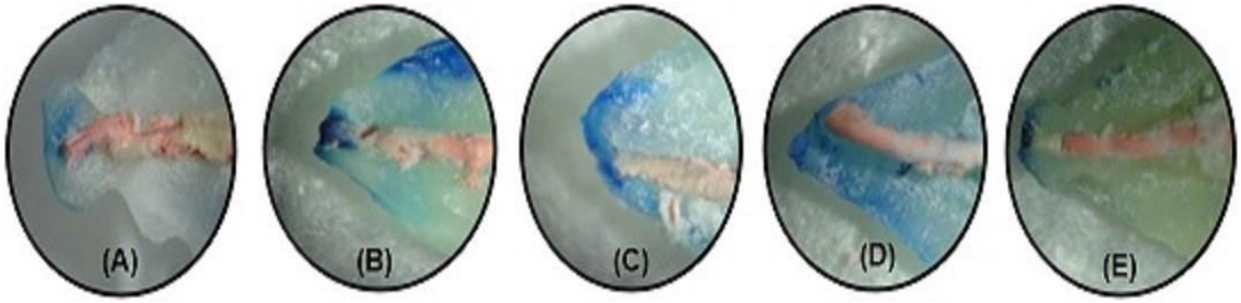

Figure 1: - [A-E] Dye penetration in each group as viewed under a stereomicroscope at 16X.

Figure 2: - [A] Tooth samples of each group immersed in 3% nitric acid for three days. [B] 2 ml supernatant taken from each sample of each group after three days for centrifugation.

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