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Comparative Study of Anti-Bacterial Effect of Modified Chlorhexidine, Chlorhexidine and Sodium Hypochlorite on *Enterococcus faecalis* Bacteria in an Experimental Study

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ABSTRACT

Sodium hypochlorite and chlorhexidine are materials that are usually used as root canal irrigants for disinfection of root canal and cleaning it. With adding surfactant sodium dodecyl sulfate modified chlorhexidine finds tissue solubility effect; thus in this study, we consider effect of ant property of this material on Enterococcus faecalis in experimental condition. Materials and methods: In this experimental study, pure colonies of Enterococcus faecalis were cultivated in BHI and then they were diluted. Next, CS 0.85% with turbidity rate of 0.5 MacFarlane was provided and then from 24 cell growth medium, 1 ml is added into each plate. 2 ml from each under tested solutions were mixed for 10 second by ultrasonic with suspension of bacteria and after certain times, then from each case, 1ml was taken and added into test tube in which fresh BHI was mixed with counteractive material of each irrigants. Finally, their turbidity rate was measured and then it was statistically analyzed. Results: There is no significant difference between chlorhexidine 1%, chlorhexidine 2%, chlorhexidine 2%, and modified chlorhexidine -0.2%. They all belong to one group. In addition, there is no significant difference between hip 5.5% and sodium hypochlorite 2.5%. However, there is significant difference. Conclusion: Results of the study indicated that using 2% SLS+ 0.2% CHXcan have similar effect as 1%-2% chlorhexidine and have 0.2% anti-bacterial effect.

Key words: Root Canal Treatment, Chlorhexidine , Anti-Bacterial Property, Sodium Dodecyl Sulfate

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INTRODUCTION

Root non-surgical treatment is predictable method of tooth maintenance which is withdrawn if the treatment is not effective. Success of root treatment with vital pulp is higher than necrotic tooth with pre-radicular lesion. This difference is due to stimulation of remained necrosis tissue and lack of ability in eliminating microorganisms and products [1]. Main factors in endodontic treatment is anatomy and form of tooth, tools, irrigants capacity and root canal obturation [1,2]. Microscopic studies indicated that root canal morphology is complicated [1, 3, 13]. Lateral canals, canals curvature, unavailable areas, and ISMOS make perfect canal cleaning impossible [1, 3, 14]. Microbial flora with necrotic root canal depends on infection stage. Firstly, there are facultative bacterial infections in the environment which survive in *aerial* and *an aerial* condition.

In non-surgical root treatment, critical need to use irrigants is ignored in process of dentistry education and then clinical works [23]. Disinfecting root canal system by preparation and

using irrigants has critical role in reducing bacteria of root canal and helping to control periapical diseases [18]. This materials complete mechanical debridement by flushing out debris, solving necrosis tissues, removing smear layer, and disinfecting root canal system [2, 8, 24]. No unique solution can perform them all completely [24].

Irrigants include endodontic material and chelating [2].

Anti-microbial property of %2CHX is similar to 5.25 NaOCl and in contrast Enterococcus faecalis is more effective [1]. One disadvantage of chlorhexidine is lack of ability in solving necrotic tissue and eliminating smear layer [1,2]. According to NaOCl disadvantages including toxicity and creating sever inflammatory reaction [3] and CHX advantages such as strong antimicrobial property, non-toxicity and biocompatibility, using them as irrigant is more appropriate [1]. According to NaOCl disadvantages and based on chlorhexidine advantages, if solubility capacity is added into chlorhexidine with specific combination, this can be suitable alternative.

Anterococcus faecalis, facultative gram positive aerial coccus is found in treated root canal with 30-60 prevalence. Treated root canals have *Enterococcus faecalis* 9 time more than first infections. This bacterium is main cause of many failures in root treatment [1, 2].

In 2011 in Rio de Janeiro of Brasil, Rôças et al performed anti-microbial effect of root canal methods by microbiologic molecule technics. They concluded that bacteria are reduced after chemical-mechanical preparation. However, certain levels of bacteria in many cases indicated that seeking effective strategies for anti-microbial treatment is necessary [37].

Poggio *et al* performed an experimental study on anti-microbial effects of different root irrigants in 2010. This study aimed at comparing anti-bacterial activities of Tetra – klein which is combined from 5.52% sodium hypochlorite, poly propylene glycol, 5.52% sodium hypochlorite niclore, 0.2% colorcsimid chlorhexidine , and cetrimide proxide hidrogene, 12 volumes in three root pathogen (*Enterococcus faecalis, Streptococcus* mutans and *Staphylococcus aureus*. Growth inhibitions of each irrigants were recorded and they were compared for each bacteria strain. Hidrogene proxide 12 volumes which are pre-heated and tetra kiln indicated highest bacteria growth inhibition [38].

Kini *et al* in 2008 performed experimental research on anti-microbial effect of 5% doxycycline, 0.2% gluconate chlorhexidine and 2.5% sodium hypochlorite which is used against Enterococcus faecalis . reults indicated that mixing 5% doxycycline and 0.2% chlorhexidine is effective irrigant against *Enterococcus faecalis* and proxide hidrogene, hase least effect in irrigation when it is used alone [39].

Estrela *et al* in 2003 in federal university of Giovanni, Brazil, studied anti-bacterial effect of 2% sodium hypochlorite and 2% Chlorhexidine by different methods. Best anti-microbial effect of NaOCI was observed in exposure test and in CHX case in distribution in agar test. Anti-microbial effect was impressed by experimental methods, biologic indices and time of exposure [40].

Gomes BP *et al* in 2001 in Campinas-UNICAMP University, Brazil, studied anti-microbial activity of different densities of sodium hypochlorite and chlorhexidine gluconate in *Enterococcus faecalis* elimination in experimental condition. Results indicated that all irrigants are effective in killing microbes in different times. In all tested densities (0.2%, 1% and 2%) and 2.25% NaOCL, chlorhexidine in liquid form was the most effective irrigants. However, required time for promoting negativity of cultivation by 0.2%col liquid and 2% chlorhexidine gel is 1 minute and 30 seconds [41].

In this study, in order to compare anti-bacterial effect of modified chlorhexidine , chlorhexidine and sodium hypochlorite on *Enterococcus faecalis* , anti-bacterial property of chlorhexidine 0.2%, 1% and 2% and modified chlorhexidine , sodium hypochlorite 2.5% and 5.25%is determined in time periods including 10-20-30-45 seconds, 1-3-5-20 minutes and anti-bacterial properties of under tested groups is compared.

Aim of this study is using modified chlorhexidine which has highest rate of sodium hypochlorite properties as an ideal irrigant as a material for irrigating canal without sodium hypochlorite , in people who are allergic to sodium hypochlorite .

MATERIAL AND METHODS

Materials and required tools

Required tools for this research included physiologic serum, blood agar, BHI, TSB, tito sulphate sodium, Tween-80, lecithin, barium chloride, sulforic acid, sodium hypochlorite, chlorhexidine 95%, modified chlorhexidine , petri dish, loop culture, cell culture, faucet with incubator, incubator oc37, digital scale, autoclave, spectrophotometry, sonicator machine, optical microscopes, stirrer heater, vortex, Sampler Tip-AHN, and sampler. In addition, bacteria strain is *Entrococcus faecalis* which is provided from fungus collection and bacteria of industrial-infection research institute in Iran.

Method

Providing bacteria suspension

In this experimental study, standard strain of *Enterococcus faecalis*, code 1394, was used. In order to provide bacteria suspension, from stock and pureed cultivation, 4-5 distict colonies were taken at most by sterile inoculation loop. They were inoculated into 4-5 ml from TSB and the environment received heat for one night in 37^0 C degree.

Process of cultivating lyophilized bacteria: in order to prepare bacteria provided from Lyophilized sample, in completely sterile condition, after breaking syringe with bacteria powder, this bacteria is entered into 5 ml of TSB and received heat for 24 hours in 37^0 C degree. After observing turbidity of environment, in order to maintain bacteria, it was transferred to blood agar cultivation area.

Providing half-macfarland: the aim of providing these standard pipes which were made by macfarland for the first time and it is used for measuring turbidity. In order to providing half-macfarland in the laboratory, 0.5 ml Barium Cloride ($BaCl_2$), 0.048 molar in liter were added into 99.5 ml normal acid sulfurinc 0.36. Accuracy of standard turbidity can be identified by spectrophotometry so when wave length is 625, absorption nanometer must be -0.1 t0 0.08. 4-6 ml standard turbidity solution was poured in the pipe with lid which is similar to a pupe that inoculation suspension is made and then the lid was closed and kept in room temperature. Standard turbidity of 0.5 macfalrland is almost $10^8 \times /_5$ bacteria in each ml and this turbidity need to be provided monthly. Using latex suspension or standards of solid status which has been explained by mcfarland standard is without problem. Microbial suspension turbidity can be put in white field with standard pipes and obtained its level from pipe number and related table.

Providing Tryptic Soy Broth: in order to compare bacteria growth with mac farland pipe 4 standard loop were cultivated in TSB while appropriate density of bacteria was obtained by comparing turbidity of environment with bacteria with macfarland pipe.

Providing BHI: in order to provide BHI, 37 g of cultivation area was solved in 1 liter stilled water then it was heated in order to make the environment integrated. After that the environment was sterile for 15 minutes at 121^0C autoclave. After cooling, it was distributed in bacteria cultivation plates and it put in incubator 37 degree for 24 hours.

Providing blood agar environment: in this study, blood Agar powder-Merk was used. 40 gram cultivation powder was solved in 1 liter stilled water and it was sterile in 121 cantigrad autoclave for 15 minutes. When growth medium temperature was 45-50, 5-8% fresh blood was added to the environment and it was stirred in 80^0 c for 10 minutes until color of cultivation area became chocolate color. Then we distribute it in still plates.

Experimental method

In first stage, in order to preparing Lyophilized sample in sterile condition, bacteria powder is entered into TSB environment and it was heated for 24 hours in 37^0 degree. After observing grown colonies in cultivation area, some *Enterococcus faecalis* colonies were diluted by physiologic serum in order to attain turbidity equals to 0.5 macfarland which consists of 1.5×10^8 bacteria in each colony. So standard suspension was provided from *Enterococcus faecalis* equals to half-macfarland. After that 1 ml provided bacteria suspension was poured in the end of sinks of cell plate and it was used as control group from sterile saline solution.

In this experiment for each under study group with certain density of irrigant solution and based on certain times and 3 repetitions from each group 24 sinks are determined.

Experimental solutions included: Chlorhexidine 0.2-1-2% and modified chlorhexidine and sodium hypochlorite 2.5 and 5.25%. For each group of irrigants solutions, 2 ml bacteria suspension was mixed with 2 ml irrigant and the it was mixed for 10 second in ultrasonic machine, then 1 ml sonic solution was added to 1 ml suspension into sink and after end of certain times 10-20-30-45 seconds and 1-3-5-20 minutes, 1 ml solution of each sink was added to 2 ml BHI along with relevant counteraction of each irrigant and then sample were incubated for 7 days in 37^0 degree. Finally after 7 days, turbidity rate of pipes is read by spectrophotometry and compares with turbidity of control sample.

Statistical method

In this study, dependent variable has anti-bacterial properties of canal irrigant solution based on which turbidity rate is measured by the machine and its scale is rational. In addition, independent variable is the type of irrigant which is used by commercial name. Scale of this variable is sodium hypochlorite 2.5% and 5.52%, chlorhexidine 0.2 and 0.1 and 2% modified chlorhexidine .

For conducting research about repetitive sizes analysis with two factors including time and different groups (density and different materials, descriptive statistical table was used for research variable.

RESULTS

In diagram 1, skewness and outlier data are indicated. Firstly outlier data need to be verified. Since outlier data have significant effect on mean and variance. In addition, based on data skewness it is better to used data logarithm because logarithm helps to variance and data skewness.

Diagram of data logarithm is drawn. Problem of skewness and outlier data solved. As it is clear in diagram 2, considering median, irrigants number 1-4 (col 1%, chlorhexidine 2% and chlorhexidine 0.2% and modified chlorhexidine 0.2%) are almost similar. In addition, turbidity median in irrigants 5 and 6 which are sodium hypochlorite 5.5% and sodium hypochlorite 2.5% are equals and more than other 4 irrigants.
As it is clear in diagram 3, turbidity rate in 10 seconds, 20 seconds and 30 seconds are very different with other times.

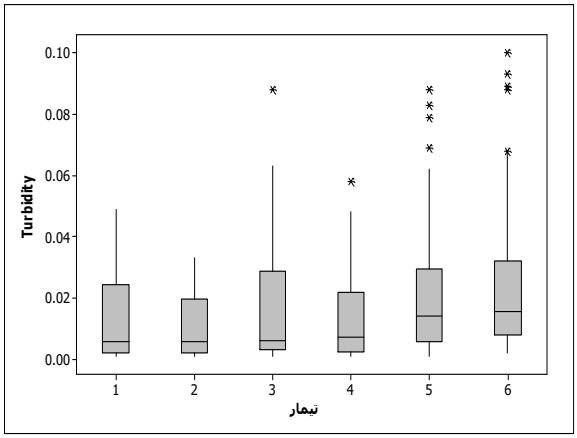

Diagram 1: turbidity boxplot in each irrigants

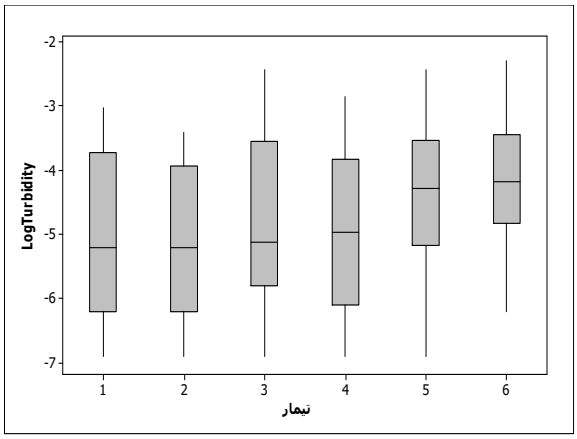

Diagram 2: turbidity logarithm boxplot in each irrigants

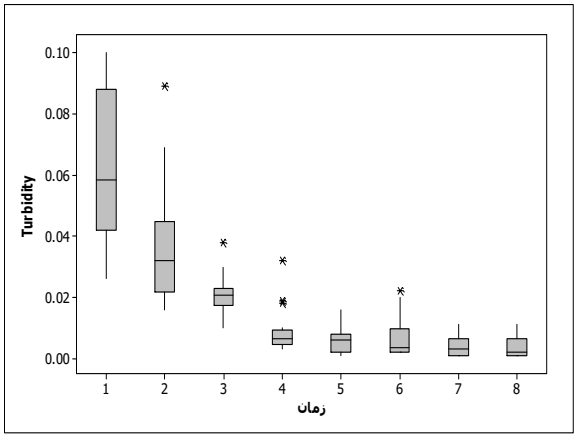

Diagram 3: turbidity boxplot in each times

Descriptive statistic related to irrigants and times are indicated in table 1. For descriptive statistic, mean central index and dispersion index of standard deviation were obtained.

Table 1: mean and deviation of irrigants

Standard deviation	Mean	Variables	
.003069	.01321	Chlorhexidine 1%	Irrigant
.002042	.01017	Chlorhexidine 2%	
.004755	.01821	Chlorhexidine 0.2%	
.003507	.01492	Midified chlorhexidine 0.2%	
.005738	.02521	Sodium hypochlorite 5.5%	
.006531	.02913	Sodium hypochlorite 2.5%	
.005737	.06161	10 seconds	Time
.004943	.03667	20 seconds	
.001523	.02067	30 seconds	
.001739	.00889	45 seconds	
.000976	.00572	1 minutes	
.001474	.00650	3 minutes	
.000783	.00389	5 minutes	
.000837	.00383	20 minutes	

Table2- Results of kolmogorov smirnov test for data normality

Turbidity	Z kolmogorov smirnov	2.672
	P– value	**0.000
Turbidity logarithm	Z kolmogorov smirnov	1.120
	P– value	.163

Table 3: variance analysis

P – value(Sig)	Mean square	Total square	Degree of freedom	Source
**.000	4.624	23.122	5	Irrigant
**.000	24.984	174.885	7	Time
	.340	44.511	131	Error

** *this indicates that significant difference is 1%*

Table 4: Duncan test

Mean		
.01321 a	Chlorhexidine 1%	Irrigant
.01017 a	Chlorhexidine 2%	
.01821 a	Chlorhexidine 0.2%	
.01492 a	Modified Chlorhexidine 0.2%	
.02521 b	sodium hypochlorite 5.5%	
.02913 b	sodium hypochlorite 2.5%	
.06161 f	10 second	Time
.03667 e	20 second	
.02067 d	30 second	
.00889 c	45 second	
.00572 b	1 minute	
.00650 b	3 minute	
.00389 a	5 minute	
.00383 a	20 minute	

In order to performing correlation analysis and variance analysis, variable normality is studied. And if it is not normal it will make normal by appropriate change.

Based on table 2, p value is less than 0.01 for turbidity. So, null hypothec is not approved.

Table2- results of kolmogorov smirnov test for data normality.

In other word, data are not normal but data normality is not rejected for turbidity logarithm ($p>0.05$).

In this section, irrigants and time are compared considering turbidity logarithm. for this, we used two way variance analysis was sued and data were analyzed. Due to abnormality of remained data from variable analysis, logarithm transformation was used. In order to make sure, most results

were compared with results of non parametric tests such as Kruskal–Wallis. Results of both methods are compatible. In addition, for pairwise comparing means, stations and seasons were measured by Duncan test. Results are indicated in table below.

Table 3 indicates that there is significant difference between irrigants and time is 1% level. In table of variance analysis indicated that irrigants and times are significantly different considering variables of the study. Since there are 6 irrigants and 8 times we specified significant difference between the time by Duncan test.

Common opinions indicates that there is no significant difference at 5% level. Based on Duncan test, there is no significant difference between Chlorhexidine 1%, Chlorhexidine 2% and Chlorhexidine 0.2% and modified Chlorhexidine 0.2 ($P>0.05$). So these irrigants are placed in one group (first group). In addition, in 5% level, there is no significant difference between sodium hypochlorite 5.5% and sodium hypochlorite 2.5% ($p>0.05$) (second group). However there is significant difference between irrigants of both groups ($p<0.05$) common opinions prove that in 5% level there is no significant difference.

Explanations; here time are classified in 4 groups

DISCUSSION

In this study, *Enterococcus faecalis* was selected for the experiment because they are first etiologic factor in pulp lesion and bacteria periapical [2, 4, 15-17]. Although 500 bacteria are identified in mouth area but less numbers can be colonized in root canal [18]. First and the main aim in endodontic treatment is eliminating microorganism from root canal system completely and providing an environment for restoring periapical tissues [2, 6].

As this study, Poggio *et al* study [40], Kini [39] *et al* and Gomes *et al* [41] and Estrela *et al* [40] studied anti-bacterial effect of irrigants on *Enterococcus faecalis* bacteria in experimental condition.

In addition to mentioned bacteria (streptococcus mutans and Staphylococcus aureus strains), in Poggio *et al* [38] study and in Estrela et al [40] S*taphylococcus* aureus, *Enterococcus faecalis*, p*seudomonas,* bacillus subtilis*, c*andida albicans, and their mixture were studied. In D'Arcangelo *et al* [42], *Fusobacterium nucleatum*, Prevotella *melaninogenica, porphyromonas gingivalis were studied.*

Gomes BP et al, similar to This study performed an experiment to study different densities of sodium hypochlorite and chlorhexidine gluconate in eliminating *Enterococcus faecali*.

In this study, different densities of sodium hypochlorite (0.5%, 2.5% and 5.25%) and two forms of chlorhexidine gluconate (gel and liquied) in 3*3 density (0.2%, 1% AND 2%) were used. There is no consensus on ideal density of sodium hypochlorite that is going to be used in root canal treatment [27]. Different densities of this material from 0.5% to 5.25% are applicated in root treatment [33, 4, 18, 27]. But common density is 2.5% in which tissue solubility and antimicrobial characteristic are maintained. This density are used in teeth with necrotic pulp or apical-periodontitis [1, 4].

One study showed that endodontists use 2%CHX more [20, 27]. It has been recommended that using 2.5% sodium hypochlorite and 0.2% Chlorhexidine simultaneously has higher anti bacteria effect than using them separately [25]. This material has better effect in 0.2$ density on bactericide than 2%density.

In Gomes BP et al study [41] 1% and 4% sodium hypochlorite densities are used but in it was more effective irrigant in 5.25% NaOCl. In Rôças *et al* study, 2.5% sodium hypochlorite was 7days mixture of medicine with sodium hypochlorite paste in both Glisirine (CHG) or paranamo chlorophyll with camphor of glisirine (CHPG).

In Estrela *et al* study, anti bacterial effect of 2% hippo and Chlorhexidine 2% was studied [40]. In SANDYA KINI et al study [39] irrigants included 5% doxycycline, 0.2% chlorhexidine gluconate, and 2.5% sodium hypochlorite were used. In Poggio *et al* study [38] and D'Arcangel *et al* cetrimide peroxide 12 volume hidrogene was used besides mentioned materials.

CONCLUSION

This study indicated that, in canal irrigation, using 2% SLS+0.2% CHX can have effect similar to chlorhexidine with 1%, 2% and 0.2% densities

and there is no significant difference ($p>0.05$). While considering anti-bacterial property, there was significant difference between chlorhexidine with different densities and modified chlorhexidine in one group with 5.5% sodium hypochlorite and 2.5% sodium hypochlorite in other group ($p<0.05$).

REFERENCES

1. Johnson W T, Noblett W C. Cleaning & shaping. In: Torabinejad M, Torabinejad M. Principles & practice endodontics. 4th ed. 2008: 287-315.
2. Kandaswamy D, Venkateshbabu N. Root canal irrigants. J Conserv Dent. 2010; 13(4):256-264.
3. Karale R, Thakore A, Shetty VK. An evaluation of antibacterial efficacy of 3% sodium hypochlorite, high-frequency alternating current and 2% chlorhexidine on Enterococcus farcalis: An in vitro study. J Conserv Dent. 2011; 14:2-5.
4. Guerreiro-Tanomaru JM, Morgental RD, Faria-Junior NB, Berbert FLCV, Tanomaru-Filho M. Antibacterial effectiveness of Peracetic Acid and conventional endodontic irrigants. Braz Dent J. 2011; 22(4): 285-287.
5. Krithikadatta J, Indira R, Dorothykalyani AL. Disinfection of dentinal tubules with 2% chlorhexidine, 2% metronidazole, bioactive glass when compared with calcium hydroxide as intracanal medicaments. JOE. 2007; 33(12): 1473-1476.
6. Baumgartner JC, Johal S, Marshall JG. Comparison of the antimicrobial efficacy of 1.3% NaOCl/ biopure MTAD to 5.25% NaOCl/ 15% EDTA for root canal irrigation. JOE. 2007; 33(1): 47-51.
7. Mehrvarzfar P, Saghiri MA, Asatourian A, Fekrazad R, Karamifar K, Eslami G *et al*. JOS 2011; 53(3): 355-360.
8. Saber S, Hashem A. Efficacy of different final irrigation activation techniques on smear layer removal. JOE. 2011; 37(9): 1272-1275.
9. Beltz RE, Torabinejad M, pouresmail M. Quantitative analysis of the solubilizing action of MTAD, sodium hypochlorite, and EDTA on bovine pulp and dentin. J Endod. 2003; 29(5): 334-337.
10. Aulton M, Taylor K. Aulton's Pharmaceutics: The Design and Manufacture of Medicines. 4th ed. 2008.537-540.
11. J. Kleier D, E. Averbach R, Mehdipour O. The Sodium Hypochlorite Accident: Experience of Diplomates of the American Board of Endodontics. JOE. 2008;34(11):1346-50.
12. Tirali R E, Bodur H, Sipahi B, Sungurtekin E. Evaluation of the antimicrobial activities of chlorhexidine gluconate, sodium hypochlorite and octenidine hydrochloride in vitro. Aust Endod J. 2013; 39: 15–18.
13. Torabinejad M, Khademi A A, Babagoli J, Cho Y, Johnson W B, Bozhilov K et al. A New Solution for the Removal of the Smear Layer. JOE. 2003; 29(3): 170-175.
14. M. Sassone L, Fidel RAS, Murad CF, Fidel SR, Hirata Jr R. Antimicrobial activity of sodium hypochlorite and chlorhexidine by two different tests. Aust Endod J. 2008; 34: 19–24.
15. Martin H. Cleanliness, disinfection, and sterilization of the root canal. Curr Opin Dent. 1991;1(6):734-436.
16. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. Int Endod J. 2009;;42(4):288-302.
17. Sadeghi, M., Thomassie, R., & Sasangohar, F. "Objective Assessment of Functional Information Requirements for Patient Portals". Proceedings of the Human Factors and Ergonomics Society Annual Meeting, 2017; 61(1), 1788-1792.
18. Iqbal A. Antimicrobial Irrigants in the Endodontic Therapy. International Journal of Health Sciences. 2012; 6(2): 153-158.
19. Zehnder M, Grawehr M, Grawehr G, Waltimo T. Tissue-dissolution capacity and dentin-disinfecting potential of calcium hydroxide mixed with irrigating solutions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003; 96: 608-612.
20. Zehnder M. Root Canal Irrigants. JOE. 2006; 32(5):389-98.
21. VS Hariharan, B Nandlal, KT Srilatha. Efficacy of various root canal irrigants on removal of smear layer in the primary root canals after hand instrumentation: A scanning electron microscopy study. J Indian Soc Pedod Prev Dent. 2010; 26(4): 271-277.

22. Siqueira J F, Rôças I N, Favier I A, Lima K C. Efficacy of Instrumentation Techniques and Irrigation Regimens in Reducing the Bacterial Population within Root Canals. JOE. 2009; 27(7): 236-237.
23. Sadeghi, M., Thomassie, R., & Sasangohar, F. "Objective Assessment of Patient Portal Requirements". Proceedings of the International Symposium on Human Factors and Ergonomics in Health Care, 2017; 6(1), 1-1.
24. Rossi-Fedele G, Doğramac E J, Guastalli A R, Steier L, Poli de Figueiredo J A. Antagonistic Interactions between Sodium Hypochlorite, Chlorhexidine, EDTA, and Citric Acid. JOE. 2012; 38(4): 426-431.
25. E. Vianna M, P. F. A. Gomes B. Efficacy of sodium hypochlorite combined with chlorhexidine against Enterococcus faecalis in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009; 107:585-589.
26. Paudel K R, Jaiswal A, Parajuli U, Bajracharya M. Different pharmacological solutions in intracanal irrigation. Nepal Med Coll J. 2011; 13(2): 111-114.
27. Parirokh M, Jalali S, Haghdoost AA, Abbott JV. Comparison of the Effect of Various Irrigants on Apically Extruded Debris after Root Canal Preparation. JOE. 2012; 38(2): 196–199.
28. Clarkson RM, Moule AJ, Podlich H, Kellaway R, Macfarlane R, Lewis D, Rowell J. Dissolution of porcine incisor pulps in sodium hypochlorite solutions of varying compositions and concentrations. Aust Dent J. 2006;51(3):245-51.
29. Effendy I, I. Maibach H. Surfactants and experimental irritant contact dermatitis. Contact Dermatitis. 1995;33(4):217–225.
30. Sajadi tabassi S.A, Mamaghani sani D. Investigation of the effects of ionic surfactants on biological membranes using human erythrocytes as a model. Contact Dermatitis. 1996;35(4):253–256.
31. Iman-del K., Mesdaghi-Nia A., Vatan-Doost S. H. Evaluation of Fungicidal Power of Dissolving Benzalkonium Chloride in Iran, Journal of the Health Association, 1996; Vol 4, pp 1-8
32. Khanade, K., Sasangohar, F., Sadeghi, M., Sutherland, S., & Alexander, K. "Deriving Information Requirements for a Smart Nursing System for Intensive Care Units". Proceedings of the Human Factors and Ergonomics Society Annual Meeting, 2017; 61(1), 653-654.
33. Marple B, Ronald P, Benninger M. Safety review of benzalkonium chloride used as a preservative in intranasal solutions: An overview of conflicting data and opinions. Otolaryngology–Head and Neck Surgery. 2004; 130(1):131-41.
34. Lee HL, I. Maibach H. Sodium Lauryl Sulfate. Contact Dermatitis. 1996; 35(4):257–267.
35. Shokri, J., Jalali, B. M., Nokhodchi, A., Adibnia K., Asli, M. M. The effects of sodium lauryl sulfate on the dissolution rate of diazepam in solid dispersions prepared by the adjacency method, Journal of Pharmaceutical Sciences, Faculty of Pharmacy, Tabriz University of Medical Sciences, 2003; pp 53-60
36. Neppelberg E, Costea DE, Vintermyr OK, Johannessen AC. Dual effects of sodium lauryl sulphate on human oral epithelial structure. Experimental Dermatology. 2007; 16:574–579
37. Rôças IN, Siqueira JF Jr: In vivo antimicrobial effects of endodontic treatment procedures as assessed by molecular microbiologic techniques.J Endod. Mar Rio de Janeiro, Brazil. 2011; 37(3):304-10. Epub Dec 30
38. Poggio C, Colombo M, Scribante A, Sforza D, Bianchi S. In vitro antibacterial activity of different endodontic irrigants. Dental Traumatology. 2012;28(3):205-9.
39. Kini S, Kundabala M, Ballal V, Shenoy R. In vitro evaluation of the antimicrobial efficacy of 5% Doxycycline, 0.2% Chlorhexidine gluconate and 2.5% Sodium hypochlorite used alone or in combinations against *Enterococcus faecalis.* Endodontology. 2011:32-6.
40. Estrela C, Ribeiro RG, Estrela CR, Pécora JD, Sousa-Neto MD. Antimicrobial effect of 2% sodium hypochlorite and 2% chlorhexidine tested by different methods: Braz Dent J Brazil. 2003; 14(1):58-62.
41. Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis* Int Endod J.2011; 34(6):424-8.

42. D'Arcangelo C, Varvara G. A comparative in-vitro study of the bactericidal efficacy of sodium hypochlorite and chlorhexidine gluconate plus cetrimide on root canal anaerobic bacterial flora. Minerva Stomatologica. 1998;47(9):381-6.