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The role of the IDJ is to inform its readers of ideas, opinions, developments and key issues in dentistry - clinical, practical and scientific - stimulating interest, debate and discussion amongst dentists of all disciplines. All papers published in the IDJ are subjected to rigorous peer review.

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Comparative Evaluation of Effect of Irrigation Solutions with Various Exposure Time on Microhardness of Root Canal Dentin (In Vitro Study)

Hanaa Abdul Jabbar Saleh

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ABSTRACT

Background: It is important to test the effect of the irrigating solutions on dentin, as they may come in contact during irrigation procedures. These irrigants cause alterations on dentin and enamel surfaces and affect their interactions with materials used for obturation and coronal restorations. The aim was to study the microhardness of root canal dentin after irrigation with different irrigant solutions for different periods.

Materials and Methods: Twenty five newly extracted non carious human permanent incisors were sectioned at cementoenamel junction and splitted longitudinally then divided into five groups; Gr1 (control) distilled water, G2: 5.25% sodium hypochlorite (NaOCl) for (10 min) then 17% EDTA for (1 min), G3: 5.25% sodium hypochlorite (NaOCl) for (10 min) then 17% EDTA for (5 min), G4: 5.25% sodium hypochlorite(NaOCl) for (20 min) then 17% EDTA for (1 min) and G5: 5.25% sodium hypochlorite(NaOCl) for (20 min) then 17% EDTA for (5 min). Vickers microhardness was evaluated.

Results: Data were analyzed using one-way ANOVA and paired t-test. The results indicated that all treatment time with 5.25% NaOCl and 17% EDTA decreased dentin microhardness significantly compared to distilled water (control). There were significant differences ($P<0.001$) between the tested groups with increasing time of exposure of irrigation solutions. Treatment with distilled water (control) showed significantly the highest microhardness value, while 5.25% sodium hypochlorite for 20 minute followed by 5 minutes (G5) with 17% EDTA showed significantly the least microhardness value followed by G4, G3 and G2.

Conclusions: Increasing irrigation time with both 5.25% sodium hypochlorite and 17% EDTA decreased dentin microhardness.

Key words: Sodium hypochlorite, EDTA, microhardness, exposure time.

تقييم مقارن لتاثير محاليل الغسل في فترات مختلفة على الصلادة الدقيقة لعاج قناة الجذر (دراسة مختبرية)

هناء عبد الجبار صالح

بكالوريوس طب وجراحة الفم والاسنان- ماجستير معالجة الاسنان/كلية طب الاسنان/جامعة الانبار. مدرس مساعد.

المستخلص

من المهم اختبار تأثير محاليل الغسل على العاج، لأنها تأتي في تماس مع العاج أثناء عمليات الغسل. هذه المحاليل تسبب تغيرات في سطح العاج والمينا وتؤثر على تفاعلها مع المواد التي تستخدم في حشوات الجذور وحشوات التيجان. الهدف من هذا البحث هو دراسة الصلادة الدقيقة لعاج قناة الجذر بعد غمرها بمحاليل غسل مختلفة لفترات مختلفة.

٢٥ من القواطع الدائمية بدون تسوسات مقلوعة لاسباب التهابات في انسجة اللثة استخدمت في الدراسة الحالية. تم قطع التيجان وفصل الجذور الى جزئين بصورة عمودية وتقسيمها الى خمس مجاميع:

المجموعة الاولى (الضبط):ماء مقطر,

المجموعة الثانية:٥,٢٥٪ صوديوم هيبوكلوريت (١٠دقائق) ثم ١٧٪ ثنائي أمين الإيثيلين رباعي حمض الخل (دقيقة).

المجموعة الثالثة: ٥,٢٥٪ صوديوم هيبوكلوريت (١٠دقائق) ثم ١٧٪ ثنائي أمين الإيثيلين رباعي حمض الخل (٥ دقائق).

المجموعة الرابعة:٥,٢٥٪ صوديوم هيبوكلوريت (٢٠دقيقة) ثم ١٧٪ ثنائي أمين الإيثيلين رباعي حمض الخل (دقيقة).

المجموعة الخامسة:٥,٢٥٪ صوديوم هيبوكلوريت (٢٠دقيقة) ثم ١٧٪ ثنائي أمين الإيثيلين رباعي حمض الخل (٥ دقائق).عينت معدلات الصلادة الدقيقة لسطح العينات بواسطة جهاز قياس الصلادة .

بعد تحليل النتائج احصائيا ,اظهرت الدراسة ان جميع المحاليل المستخدمة وفي جميع الفترات سببت انخفاض كبير في صلادة العاج مقارنة مع الماء المقطر. المجموعة الاولى التي تم معاملتها مع الماء المقطر سجلت اعلى قيمة للصلادة بينما المجموعة الخامسة سجلت اقل قيمة تليها المجموعة الرابعة, المجموعة الثالثة والمجموعة الثانية. الاستنتاجات المستنبطة لهذه الدراسة تشير الى ان الانخفاض في الصلادة يزداد بزيادة وقت المعالجة مع هذه المحاليل.

INTRODUCTION

Mechanical instrumentation of the root canal produces a smear layer that covers the dentinal tubules. The smear layer is an amorphous irregular layer containing inorganic dentin debris as well as organic materials like pulp tissue, odontoblastic process, necrotic debris, microorganisms and their metabolic products [1].

There is a controversy over whether to remove or maintain the smear layer, but a recent systematic review and meta-analysis of leakage studies concluded that the removal of the smear layer improves the fluid tight seal of the root canal system. It also hinders the penetration of intracanal medications and sealers into dentinal tubules [2]. Effective cleaning of the canal system requires the use of irrigation solutions during instrumentation and irrigation, which serve variety of purposes including antibacterial action, tissue dissolution, cleaning and chelating [3].

The most widely used endodontic irrigant is 0.5% to 6.0% sodium hypochlorite (NaOCl), because of its bactericidal activity and ability to dissolve vital and necrotic organic tissue [4,5]. However, NaOCl solutions exert no effects on inorganic components of smear layer. Chelant and acid solutions have been recommended for removing the smear layer

from instrumented root canals, including ethylene diaminetetraacetic acid (EDTA), citric acid and phosphoric acid [6,7]. Ethylene diaminetetraacetic acid (EDTA) is generally accepted as the most effective chelating agent in endodontic therapy. It is used to enlarge root canals, remove the smear layer and prepare the dentinal walls for a better adhesion of filling materials. The disodium salt of EDTA at 17% concentration and neutral pH is widely preferred for root canal treatment [8]. Root canal irrigation with the previously described solutions can lead to structural changes, as evidenced by the reduction of dentin strength, microhardness and changes in surface roughness [9,10]. **Baumgartner and Mader** [11] reported that when EDTA and NaOCl solutions were alternately applied to an uninstrumented root canal wall dentin showed an eroded appearance and tubular orifice diameters were enlarged. **Oliveira et al.** [12] reported that 1% NaOCl for 15 min decreased root dentin microhardness.The decalcifying effect of chelating agents depends largely on application time, solution pH and concentrations [13].

Although a reduction in microhardness facilitates the instrumentation throughout the root canal, it may also weaken the root structure [14]. Microhardness determination can provide indirect evidence for losing or gaining any mineral substance in the dental hard tissues [15].The time of exposure to the irrigants is a factor that has gained little attention in endodontic studies. Even fast-acting biocides such as sodium hypochlorite require an adequate working time to reach their potential [16].Therefore, the purpose of this study was to examine the effect of irrigant solutions that applied for different time on microhardness of root canal dentin.

MATERIALS AND METHODS

Samples selection and preparation

Twenty five newly extracted non carious human permanent incisors extracted primarily for periodontal reasons were selected for this study. The teeth were stored in distilled water after the root surface was cleaned with curettes. Teeth are examined under 20x magnification in a microscope (Langenfeld, Germany), those having cracks were eliminated to prevent false results.The pulp of all selected teeth were removed using barbed broaches [17]. Then crowns were sectioned at the cementoenamel junction by using diamond disk under water cooling.The roots then sectioned longitudinally in the buccolingual direction to obtain root halves (n=50) using diamond disk under water coolant. Root specimens were horizontally embedded in autopolymerizing acrylic resin leaving the dentin surface exposed. The surface of each root half was polished with silicon carbide paper (500, 800 and 1000 grit) (Leco, St. Joseph, USA) under constant water coolant [17].The prepared samples divided into five experimental groups(n=10):

Group 1: Distilled water (control).
Group 2: 5.25% sodium hypochlorite (NaOCl) for (10 minutes) then 17% EDTA for (1 minute)
Group 3: 5.25% sodium hypochlorite (NaOCl) for (10 minutes) then 17% EDTA for (5 minutes)
Group 4: 5.25% sodium hypochlorite (NaOCl) for (20 minute) then 17% EDTA for (1 minute)
Group 5: 5.25% sodium hypochlorite (NaOCl) for (20 minute) then 17% EDTA for (5 minutes)

All specimens received a final flush with 10 ml distilled water immediately after treatment for the determined time to avoid the prolonged effect of chelating solution and dried with sterile paper point. The same procedure was carried out after treatment with NaOCl [17].

The specimens were mounted on stage of Vickers microhardness tester. The midroot portion is halfway from the outer surfaces was focused for testing. Indentations were made with Vickers diamond indenter using 300 gm load with a dwell time of 20 second. These indentations were measured and converted into Vickers hardness number (VHN) values by the monitor [18].

RESULTS

Vickers microhardness values (means ± SD) for the irrigating regimens are summarized in (Table 1). Group1 (control) showed significantly the highest microhardness value and group5 demonstrated the least microhardness value.

Table (1): Descriptive statistics of microhardness values for all groups

Groups	*N*	*Mean*	*±S.D*	*Min*	*Max*
Group1 (control)	10	62.56	±1.03	61.10	64.25
Group2	10	54.74	±.97	53.42	56.08
Group3	10	53.62	±.99	52.33	55.15
Group4	10	51.54	±.94	49.65	53.28
Group 5	10	50.05	±.65	48.87	50.74

Data were analyzed using one-way ANOVA and paired t-test. In these tests, P>0.05 (Non significant), P<0.05 (Significant), P<0.001 (Highly significant). One-way ANOVA test demonstrated that the time of treatment with 5.25% sodium hypochlorite (NaOCl) and 17% EDTA had a significant influence on microhardness of root dentin (P<0.001).

Table (2): One-way ANOVA test

	Sum of Squares	DF	Mean Square	F	P-value.
Between Groups	947.13	4	236.78	91.92	.000 (HS)
Within Groups	115.92	45	2.58		
Total	1063.04	49			

Further analysis was done by using paired t-test to compare between each two groups with different treatment time. Comparison between group1 (control) and group2 showed significant difference (P<0.05), while the differences were highly significant between group1 and all other tested groups with increasing exposure time to both 5.25% sodium hypochlorite (NaOCl) and 17% EDTA (P<0.001).

Table(3):Paired t.test for all groups

Paired	t-value	DF	p-value	Sig.
G1 - G2	3.03	9	.014	S
G1 - G3	7.54	9	.000	HS
G1 - G4	12.86	9	.000	HS
G2 - G3	6.04	9	.000	HS
G2 - G4	8.60	9	.000	HS
G3 - G4	3.65	9	.005	HS

DISCUSSION

Irrigants used in endodontic treatment caused alterations in the chemical and structural composition of dentin during removal of smear layer [19,20]. Recently, **Uzunoğlu et al**. [21] stated that fracture resistances of root canal treated teeth were affected by irrigation procedures. Ideally, mechanical properties like strength, composition and hardness of dentin should not be affected in any negative aspect after irrigation procedures or this effect should be minimized. However, the sequential use of EDTA (or any acid) and NaOCl causes a progressive dissolution of dentin at the expense of peritubular and intertubular areas [22]. The efficacy of chemical agents used to remove smear layer and demineralize and soften root dentine during root canal treatment has been examined by various means, including microhardness measurements, micro-radiographic assessments, spectrometry studies (**Verdelis et al.**[23], **Dogan & Calt** [24], **Scelza et al.**[25], **Machado-Silveiro et al.** [26], **Ari & Erdemir 2005** [27], **Gonzalez-Lopez et al. 2006** [28]) and especially electron microscopy studies (**Calt & Serper** [29], **Di Lenarda et al.**[30], **Ayad** [31], **Haznedaroglu** [32], **Perez-Heredia et al.**[33]). The assessment of the microhardness of a material is one of the simplest nondestructive mechanical characterization methods. Hardness is measured as the resistance to the penetration of an indenter that is harder than the sample to be analyzed [34]. In our study Vickers microhardness test was used because previous studies have shown the suitability and practicability of Vickers microhardness test for evaluation of surface changes of dental tissues treated with chemical agents [15,31]. Sodium hypochlorite (NaOCl) at a higher concentration (5.25%) was more effective in disinfection of the dentinal tubules [35] so in this study we used this concentration to study its effect on root canal dentin when used as root canal irrigant.

The present study showed that all irrigation periods with 5.25% sodium hypochlorite and 17% EDTA decreased dentin microhardness significantly. Treatment with 5.25% sodium hypochlorite for 10 minutes followed by 5 minutes 17% EDTA (group3) showed a significant decrease in microhardness from control group that treated with distilled water and also significantly lower than irrigation for same treatment time with 5.25% NaOCl but for 1 minute with 17%EDTA (group2). This in accordance with other studies which reported that EDTA when used for more than 1 minute causes erosion of dentinal tubules, thus reducing the dentin microhardness and consequently causing root fragility [36,37].

A previous study showed that 17% EDTA either alone or in combination with a tensoactive cationic detergent (Cetavlon) caused a more significant reduction of root dentin microhardness than 10% citric acid [34]. **Cruz-Filho et al.** [38] reported that the action of 17% EDTA in decreasing dentin microhardness can be observed within the first minute after application of this chelator and that dentin microhardness decreases as the contact time with the solution increases.

The use of NaOCl as an initial irrigant creates an apatite-rich collagen-sparse dentin subsurface [39,40] that is more brittle than untreated mineralized dentin [41].This collagen-sparse subsurface zone has the potential to create non uniform deproteinization channels [42] that facilitate subsequent EDTA

penetration and apatite dissolution, removal of the "superficial subsurface" organic phase from the mineralized dentin by NaOCl is both concentration and time-dependent. The combined action of NaOCl and EDTA causing changes on collagen matrix and demineralization of root dentin with consequent exposure of collagen respectively results in a decrease of dentin microhardness as observed in the presented study [43].

Calt and Serper [44] studied the time-dependent effect of EDTA followed by NaOCl which can be the evidence for the dentin microhardness decrease. EDTA as a time-dependent solution after 5 min decreased dentin microhardness more than its 1 min application at a depth of 100 mm from the pulp-dentin interface. Many studies have shown that different concentrations of chelating agents and citric acid can reduce dentin hardness [17] and this effect increases with increase exposure time [22].

The result of this study demonstrated that the treatment with 5.25% sodium hypochlorite for 20 minute followed by 5 minutes 17% EDTA (group5) significantly decrease the microhardness from control group and also significantly lower than all other groups including the group treated for same time with NaOCl but for 1minute with 17%EDTA (group4). This result in accordance with **Slutzky-Goldberg et al.**[45], they used irrigation with 2.5% or 6% NaOCl for 5, 10, or 20 min without subsequent EDTA they concluded that exposure of dentin to sodium hypochlorite solution for more than 10 min decreased dentin microhardness significantly. The decrease in microhardness was more marked after irrigation with 6% NaOCl than with 2.5%. **Zhang et al.** [46] showed different concentrations of NaOCl cause a time-dependent linear increase in removal of the organic phase from mineralized dentin and the extent and rate of removal were more severe with the use of higher concentrations (5.25%) when NaOCl was used as the initial irrigant. **Saleh and Ettman** [36] studied the effect of endodontic irrigation solutions (3% H2O2 and 5% NaOCl or 17%EDTA for 60 second) on the microhardness of root canal dentin, the results showed that irrigation with H2O2/NaOCl or EDTA significantly reduced the microhardness of root dentin.

Unlike what is commonly accepted, the treatment of dentin with NaOCl may not only remove the organic matrix but also some of the inorganic content that ultimately renders dentin much weaker than normal [47]. Although NaOCl is not a chelating agent, it can significantly decrease the C (calcium)/P (phosphorus) ratio of superficial root dentin [24] and its microhardness [15] depending on the concentration of the solution.

CONCLUSION

Within the limitations of this study, irrigation of root canal with 5.25% sodium hypochlorite (NaOCl) for (20 min) followed by 17% EDTA for (5 min) resulted in decreasing of root canal dentin microhardness. So the time of irrigation is a factor that should gained special attention during endodontic treatment.

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Evaluation of The Efficacy of Diode Laser, Chlorhexidine Digluconate Gel and Calcium Hydroxide Paste In The Disinfection Of Candida Albicans Infected Root Canals, In Vitro Study

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ABSTRACT

the study aims to evaluate the efficacy of CHX-gel, $Ca(OH)_2$ and diode laser in eliminating C.albicans from the root canal system and hence to eliminate the chances of reinfection and minimize treatment time.

Materials and methods: 180 single rooted anterior teeth were prepared and divided into 4 equal groups, the first was prepared to ISO 60 while the 2nd-4th were prepared only to ISO40, all the teeth were then sterilized and inoculated with C.albicans suspensions for 14 days, followed by evaluation of the starting bacterial counts in each tooth by means of colony forming units (CFU/ml), after this the groups 2-4 were prepared to ISO 40 MAF, using NaCl 9% in the 2nd group, NaOCl 1% in the 3rd and CHX 0.2% in the 4th. Finally each of the 4 groups was divided into three subgroups (n=20), the first irradiated with diode laser, the second with injected with $Ca(OH)_2$ paste and the third injected with CHX-gel for 7 days, followed by estimation of the end bacterial counts in CFU/ml in canal lumen and in dentin.

Results : the reduction of C.albicans was higher in all the groups after $Ca(OH)_2$ and CHX gel injection than with diode laser irradiation ($p<0.001$, Chi-square test). After chemomechanical preparation a higher effectivity of the laser disinfection was shown and more specimen could reach the lower limit values, the effectiveness was also increased by the use of NaOCl or CHX as irrigants ($p<0.001$).

INTRODUCTION:

A preliminary condition for successful root canal therapy is the reduction of microorganisms before filling of the root canals (Sjogren et al. 1997, Sundqvist et al. 1998), in spite of the reduction achieved by the chemomechanical preparation and the utilization of intracanal medicaments, some microorganisms stay in the irregularities of the canals and the dentinal tubules (Peters et al. 2000) which can be a reason for minimizing success rates and reinfection (Sjogren et al. 1997) this is not only caused by individual failures but by existence of resistant bacterial species (Sundqvist 1994), usually the primary endodontic infections are associated with a mixed anaerobic population, while secondary infections are mostly caused by enterococci species or candida albicans (Waltimo et al.1997, Sundqvist et al. 1998, Pinheiro et al. 2003), the effectivity of the classic calcium hydroxide against these species has been found limited (Waltimo *et al.* 1999, Siqueira *et al.* 2004, Siren *et al.* 2004), thus many alternatives have been tried to optimize the disinfection of the root canal system and to enable a one session therapy. Laser light have antimicrobial properties and can achieve an effective microbial reduction in vivo (Moritz *et al.* 1997), till now little evidence based studies have focused on their effect in achieving effective one session therapy.

The aim of our study is to find the effectivity of laser application in comparison with the use of $Ca(OH)_2$ and CHX- gel in the reduction of C. albicans from the root canal system.

MATERIALS AND METHODS

Study design

As a part of our study three types of treatment were carried on, which required classifying the teeth into three subgroups, in the first subgroup diode laser application was done (GENTLEray 980, Kavo, Germany), in the second a 7 day intracanal medication of Calcium hydroxide paste (UltraCal XS, Ultradent, USA) was injected and in the third subgroup a 7 day intracanal medication of CHX (Chlorhexamed, 1% gel, Galaxosmithcline) was injected.

Every one of these treatments was examined alone and in combination with the other therapy methods which included mechanical preparation/ no preparation conditions and NaCl or NaOCl or CHX irrigation conditions, a total of 12 study groups were built , each study group had a total probe count of 15 specimen, so a total of 180 probes was examined.

Table.1. description of the study groups:

Subgroup (n=15)	*Main group (n=60)*			
	1	*2*	*3*	*4*
	Without preparation	*Preparation with NaCl irrigation*	*Preparation with NaOCl irrigation*	*Preparation with CHX irrigation*
Laser	1.1	2.1	3.1	4.1
$Ca(OH)_2$	1.2	2.2	3.2	4.2
CHX-Gel	1.3	2.3	3.3	4.3

n: number of teeth (probes).

Before beginning of the study sequence a complete preparation of the specimens of main group 1 was done to ISO 60(MAF) while in the groups 2-4 initial preparation to ISO 40 was done (MAF), the complete preparation of the canals in these groups was done during the study, in the main group 2 with physiologic normal saline (NaCl 1%), in the main group3 with sodium hypochlorite (NaOCl 1%) and with CHX-gel 0.2% in the 4th main group. The subgroups 3 and 4 served as positive controls, the negative controls were the bacterial counts of 1st subgroup, the following table describes in details the study procedures:

Table.2; description of the study procedure.

Main group1	*Main group 2*	*Main group 3*	*Main group 4*
Irrigation and dryness Sterilization Placement in tubes			
Preparation to ISO 60 NaCl irrigation	Preparation to ISO 40 NaCl irrigation		
Sterilisation Implantation of C.albicans Incubation			
Starting bacterial count (CFU/ml)			
No further preparation	Further preparation to ISO 60		
	NaCl irrigation	NaOCl irrigation	CHX irrigation
1.1	2.1	3.1	4.1
1.2	2.2	3.2	4.2
1.3	2.3	3.3	4.3
End bacterial count 1 (CFU/ml) End bacterial count 2 (CFU/mg Dentin)			

Preparation of the specimens:

A total of 180 single rooted extracted teeth were collected, after full debridement with ultrasound endo tips (SONICFlex, Kavo),they were sterilized with ethylene oxide and the crowns cut with diamond burs and all the specimen were brought to a length of a 19mm, Fig.1,

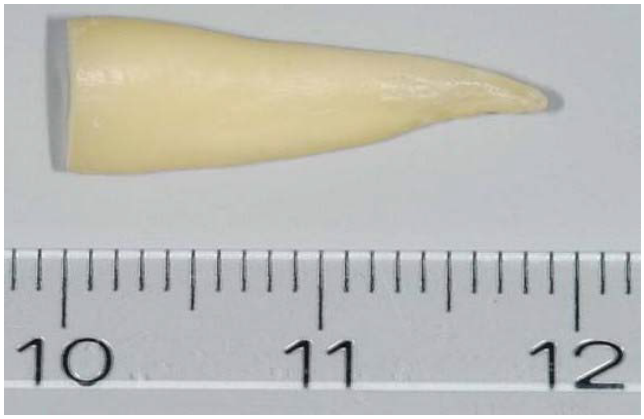


Fig.1; root length determination.

During the period of preparation the samples were kept in physiological normal saline and the preparation of all teeth was unified to a length of 18 mm. Specimen of the first main group were prepared to ISO 60 and of the other groups to ISO 40 as listed above (Flexmaster System VDW), during the preparation debris were removed using normal saline (NaCl 0.9%) and EDTA (FileEze, Ultradent) was used to wash away the smear layer. To ensure a unified preparation depth, composite stoppers were designed on the files, and the orifices of the canals were assured to be cone shaped using 2.9mm Ø, orifice former diamond (Riitano access bur kit, Ultradent, USA). To ensure ease of access. After preparation each of the specimen were kept in sterile normal saline containers for 14 days and the solution was changed everyday.

After preparation the teeth were painted with nail polish followed by embedding in fast setting resin, Fig.2,

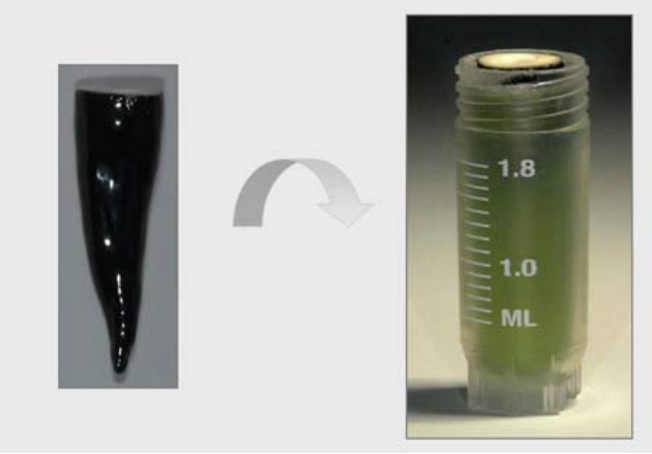


Fig.2; nail polish painting and embedding of specimen.

To ensure sterilization ten specimen were selected randomly and injected with universal nutrition solution (Tryptone Soya broth) and incubated for 14 days at 37°C the resulting solution was then implanted over columbia agar with sheep blood and incubated for 24 hrs at 37°C. after proving the sterility a 30µl suspension of C.albicans in Sabouraud Dextrose solution injected in the canals of all the teeth in the specimen, the starting concentration was 10^5 CFU/ml, the specimen was incubated for 14 days at 37°C and a sterile nutritive medium was daily given (Sabouraud liquid medium, Oxoid), the cover of the embedding tubes was kept loose. Before beginning of the main experiment To obtain the intracanal bacterial count , 5µl of the solution was drawn with paper points and with a dilution of 1:4000000 implanted, after an incubation for 24hrs at 37˚C, the resulting colonies were counted and the count of microorganisms in the canal per ml of liquid (CFU/ml) against the dilution factor was calculated.

To be able to calculate the exact bacterial count pro milliliter of solution in the canal we need to know the exact volume of fluid taken by the paper point and hence to evaluate this, paper points (ISO 40 VWD) were immersed in previously pipetted volumes of 5, 10 and 15 µl of liquid to the depth of 18 mm. we found that an average paper point can fully absorb up to 5µl of the solution. To reach the dilution value , paper points were immersed in sealable tubes with 1,995ml normal saline, the tubes were placed in vortex mixer for 30 seconds, from this solution 10µl were pipetted and placed in 0.99ml sterile normal saline tubes then vortexed for 30s, 10µl were pipetted from this solution and implanted in (Sabouraud-Glucose-Hefeextract selective media, Oxoid) and so a dilution of 1:4000000 was reached which enables us to find the exact bacterial count pro ml.

Pipetted volume	Dilution factor
5µl paperpoint in	X 400
10µl solution in 990µl NaCl	X 100
10µl solution	X 100
	= 4.000.000

Calculation of bacterial count was done on a selective media for fungi (Sabouraud- Glucose-Hefeextract selective media, oxoid), in addition three specimen from each subgroup were examined for contamination on universal nutritive media (Columbia agar with sheep blood, oxoid) during the start and the end bacterial count procedures.

Procedure:

Immediately after calculation of the starting bacterial count, the experimental procedure started with the preparation of the specimen of groups 2-4 to ISO 60 with 9% NaCl in group 2 and with 1% NaOCl in group3 and with 0.2% CHX in the 4th group. Candida solution was washed with 2ml 9%NaCl and each canal was dried with 3 paper points ISO 40, corresponding to their classification in subgroups, the specimen were treated after that as follows:

The first subgroup was irradiated with diode laser (Epic, Biolase, USA) with maximum output power of 7W,200µm fiber, inserted into 17mm length, the fiber was inserted in spiral movement along the canal wall in 3mm/s for 10s from apical to coronal, Fig.3;

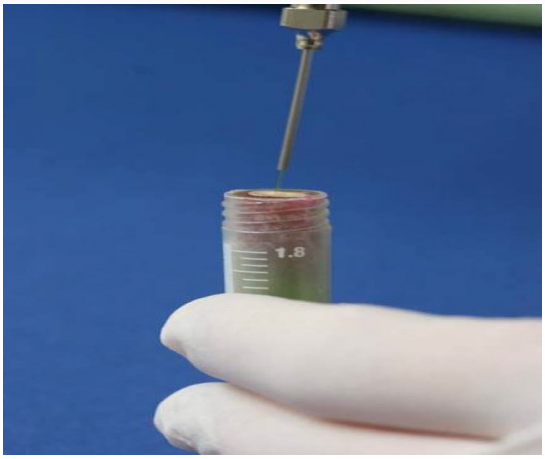


Fig.3; laser application procedure.

In each canal the procedure was repeated 4 times for overall 40s.

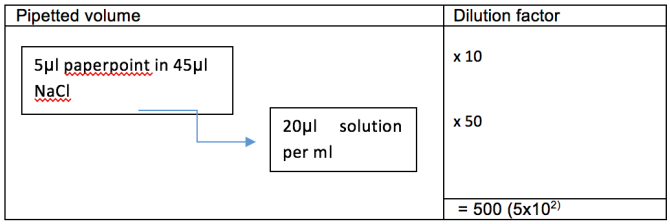
In the 2nd subgroup, chlorhexidine digluconate gel (1%) was injected in the canals and they were incubated for 7 days, a disposable endo injection tip was used (NaviTip, Ultradent, USA) to inject the material slowly from apical to coronal to avoid airbubble formation and adequately fill the canal then canals were covered and incubated for 7 days at 37°C. Similarly in the 3rd subgroup the canals were injected with calcium hydroxide paste (UltraCal XS(Ph 12.5), Ultradent, USA) with endo tips (NaviTip, Ultradent, USA) from apical to coronal in a slow movement then covered and incubated for 7 days at 37°C.

To obtain the end bacterial count, all canals were washed with 2 ml NaCl (0.9%) solution. In the first subgroup this was done after laser irradiation while in the 2nd and 3rd subgroups after the incubation, then paper points (ISO 40) were used to take the bacterial specimen (CFU/ml) and Hedstrom files (ISO 40) were used to obtain dentinal specimen from the root canal wall (CFU/mg).

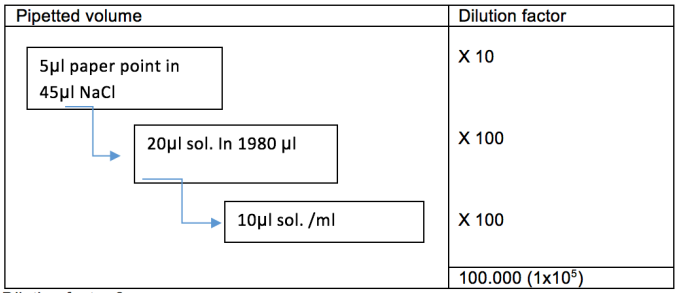
Evaluation of the end bacterial count:

a.End bacterial count of the canal :

Because of the previously investigated C.albicans growth sequence in literature , the dilution factor and the starting average bacterial count could be evaluated so that an average bacterial count of 10^8 CFU/ml could be accepted and the dilution factor of 1:4000000 was considered (Sen et al. 1999-2000, Valera et al. 2001, Ferguson et al. 2002, Siqueira et al. 2003, Menezes et al. 2004, Brandle et al. 2008) . For the end count this was not possible since we have no expected value for the CFU/ml. In case we consider a smaller dilution factor so the result is higher number of colonies >1000 which are difficult to count and their growth behavior is limited on the media, a smaller dilution however gives the possibility to lower the lowest confirmed bacterial count to $5x10^2$ CFU/ml, so to keep the lowest limit as small as $5x10^2$ and to have the possibility to quantify specimen with higher bacterial concentrations, 2 dilution factors were produced and implanted so that any concentration from $5x10^2$ to 10^8 CFU/ml could be quantified.



Dilution factor 1



Dilution factor 2

b.Evaluation of the end bacterial count of the canal wall dentin:

ISO 40 Hedstrom file was inserted 3 times along the canal wall to obtain the dentinal specimen, files were then placed in sealable tubes (Safeseal Microtube 2ml, Germany) with 50 µl sterile normal saline and vortexed for 30s then 20µl were pipetted and implanted on (Sabouraud-Glucose-Hefeextract, Oxoid) selective media. After an incubation for 24hrs at 37°C colonies were counted. First 10 files with and without dentin specimen were weighted and an average 0.0003g specimen weight was obtained. Since 20µl were implanted so multiplying the counted colonies by 2.5 gives the bacterial count in 50µl, this corresponds the count in a 0.0003 g specimen and the division on 0.3 gives the count per milligram (CFU/mg).

Statistical analysis:

For the quantitative evaluation, three variables were considered:

a.End bacterial count 1 (end bacterial count in canal) CFU/ml

b.End bacterial count 2 (end bacterial count in dentin) CFU/mg

Then all the values of the starting bacterial count were considered as baseline and tested with Kruskal-

Wallis test for difference (p <= 0.05). since the end bacterial count inside the same subgroup varied either below or above the limits, we built three classes for the end count values , the classes were (under the lower limit, middle value , high value), presence of these classes for all the 12 subgroups was summarized in cross tables and graphs were produced for each main group. The study groups were compared concerning the end bacterial count in canal and in dentin, each single treatment group against different preparations and different preparation against treatments were compared with Chi-square test, all the previous tests were carried out using SPSS software 16.0 (SPSS, Germany). 2 factor ordinal logistic regression analysis were carried out to evaluate the three levels of end bacterial count and the relative bacterial count reduction using the software SAS 9.1 (SAS Institute, USA). The start and end bacterial count in dentin was compared (Chi-Quadrat-test) and the presence of a relation between the end bacterial count in canal and in dentin was examined.

RESULTS

Quantitative evaluation:

Before starting the experimental work, a random specimen of the experimental teeth starting from 10^5 CFU/ml, could reach a concentration of 10^8 CFU/ml after 14 days, in this power the concentration remained until 21 days, so the incubation period of C.albicans suspension was fixed on 14 days.

Starting bacterial count:

At the beginning of the procedure the starting count was estimated in all the canals, the median value was $3.66x10^8$ CFU/ml, where the lowest value was $8x10^6$ and the highest $1.28x10^9$ CFU/ml. the starting count in dentin was estimated by selecting 4 more canals , implanting them with C.albicans and incubation for 14 days, the starting counts in the canals and in the dentinal wall were:

Table 4; starting bacterial counts in the canal and in the dentin of the selected specimen.

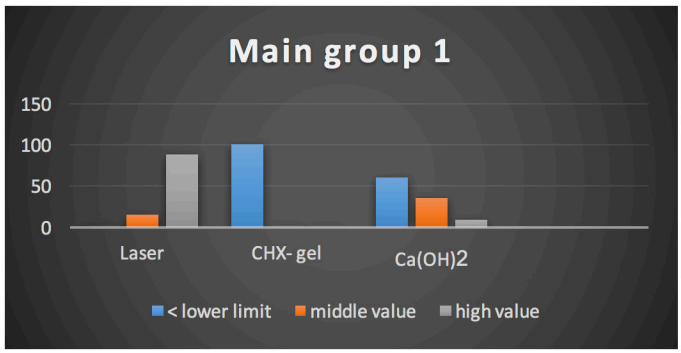
Specimen	*Starting count in canal*	*Starting count in dentin*
1	1 3,84 × 10^8 CFU/ml	7,10 × 10^3 CFU/mg
2	2 2,04 × 10^8 CFU/ml	1,02 × 10^5 CFU/mg
3	3 9,60 × 10^8 CFU/ml	9,83 × 10^4 CFU/mg
4	4 2,56 × 10^8 CFU/ml	6,80 × 10^4 CFU/mg
Average	4,51 × 10^8 CFU/ml	8,48 × 10^4 CFU/mg

End bacterial count 1:

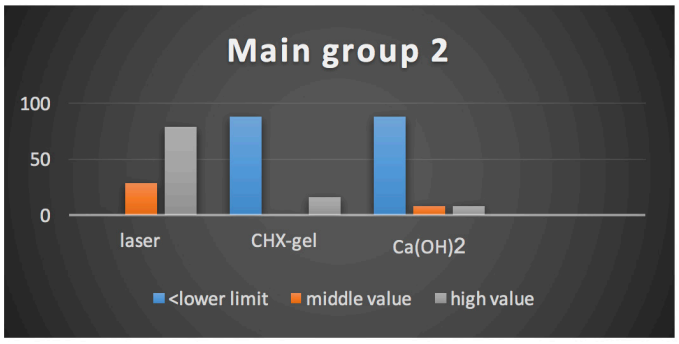
The end bacterial count was estimated in all the specimen and since the values varied but either were lower than a lower limit or higher than an upper limit, 3 classes of values were considered for statistical analysis.

Lower than the lower limit	< 5 × 102 CFU/ml
Middle value	5 × 102 - 4 × 103 CFU/ml
High value	> 4 × 103 CFU/ml

Classification of the end bacterial counts in the value fields was done for all the specimen and the results were tested against one another (Chi square test, α= 0.05), to simplify the results, percentages (of 100%) of each value were clarified using bar charts for each main group:



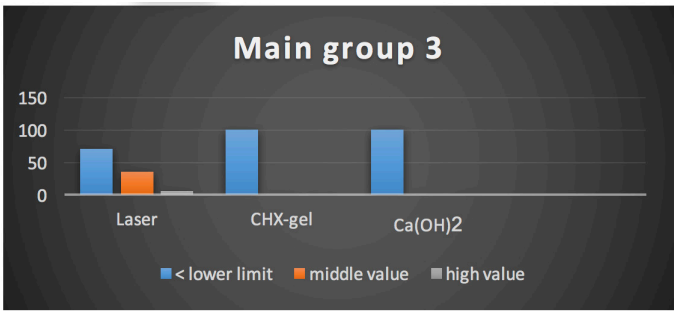
By the use of $Ca(OH)_2$, 60% of the cases were in the lower limit field and 33,3% were in the middle field, using diode laser no values were in the lower value field and 86,7% were in the high value field, the utilization of Ca $(OH)_2$ had significant effect from the laser treatment ($p<0.001$) and the use of CHX-gel caused significant decrease more than $Ca(OH)_2$, ($p=0.017$) were all the specimen were in the lower value field. .



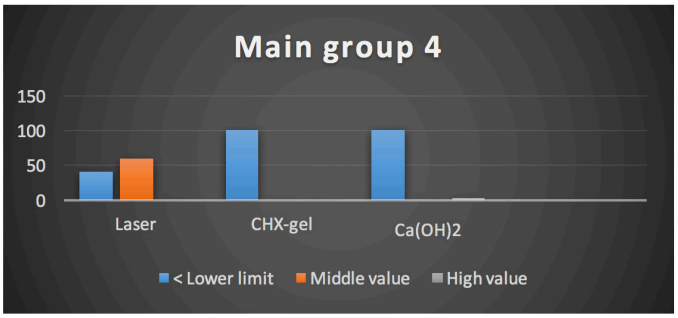
through the additional preparation and use of NaCl the effect was clear on having specimen with lower limit values for the laser and $Ca(OH)_2$ groups, utilization of $Ca(OH)_2$ and CHX-gel did not differ significantly , both reached 86,7% bacterial count under the lower limit, while laser treatment did not have any counts below the lower limit.

Utilization of NaOCl as irrigant in group 3 , caused an increase in the number of teeth without C.albicans after laser and $Ca(OH)_2$, only the laser

group showed values higher than the high value. The combination of chemomechanical preparation and NaOCl irrigation together with CHX-gel or $Ca(OH)_2$ resulted in bacterial count reduction below the lower limit in all the treated teeth, however there was a significant difference between CHX-gel and $Ca(OH)_2$ from the laser therapy (p=0.042).



In group 4 the chemomechanical preparation with CHX-irrigant resulted in more specimen in the subgroups of laser and $Ca(OH)_2$ below the lower limit, this was clear for about 70% of the laser and 100% of $Ca(OH)_2$ specimen.



The results show that the lower limit values of < $5x10^2$ CFU/ml could be 100% achieved through application of CHX gel without further preparation using CHX irrigant, NaCl or NaOCl.

End bacterial count 2:

Since the values varied they were classified in three value fields according to the following tables:

Table.6. classification of the end bacterial count 2 values in main group 1;

No preparation (CFU/mg)	*preparation*			
	Laser	*CHX-gel*	*$Ca(OH)_2$*	*Total*
< lower limit	0	15	11	26
8,33-4,999	0	0	4	4
> 5000	15	0	0	15
Total	15	15	15	45

Table.7. end bacterial count 2 values in main group 2;

NaCl (CFU/mg)	*preparation*			
	Laser	*CHX-gel*	*$Ca(OH)_2$*	*Total*
< lower limit	0	15	13	28
8,33-4,999	1	0	2	3
> 5000	14	0	0	14
Total	15	15	15	45

Table.8. end bacterial count 2 values in main group 3;

NaOCl (CFU/mg)	*preparation*			
	Laser	*CHX-gel*	*$Ca(OH)_2$*	*Total*
< lower limit	11	15	15	41
8,33-4,999	4	0	0	4
Total	15	15	15	

Table.9. end bacterial count 2 values in main group 4;

CHX (CFU/mg)	*preparation*			
	Laser	*CHX-gel*	*$Ca(OH)_2$*	*Total*
< lower limit	11	15	15	41
8,33-4,999	3	0	0	3
> 5000	1	0	0	1
Total	15	15	15	45

DISCUSSION

We examined C.albicans in our research as one of the most causative factors of endodontic revisions (Siqueira et al.2004). After a standatdised procedure the starting situation could be unified in all the probes, the standard deviation ($2,728x10^8$) was due to difference in dentin morphology between the teeth and was considered biologically unnoticeable since the average value of all the probes had an exponent of 10^8. The starting bacterial count in our specimen as average of $4,51 \times 10^8$ CFU/ml is near to that found as an average count of $4x10^5$ in canals by Bystrom (Bystrom et al. 1981) and that in dentin of $8,48 \times 10^4$ CFU/mg is somewhat lower than that found in infected canals by Peters $5x10^6$(Peters et al. 2001). The comparision of the end bacterial count1 to the starting counts showed an indication for the treatment, in main group 1 the treatment with CHX-gel was not significantly different from that of $Ca(OH)_2$,(α<= ٠,٠٠٤٢, Chi square test) they both were significantly different from the laser group. More frequently there was a significant difference between the CHX-group

and the laser group ($p<0.001$), the results were similar for $Ca(OH)_2$ as the bacterial reduction was frequently higher than that of the laser group ($p<0.001$). considering the bacterial count reduction in dentin the results were similar to the previous and the reduction was more by CHX-gel then $Ca(OH)_2$ then laser. The end bacterial counts of the free (canal) and adherent bacteria are highly related to this sequence as laser irradiation has not the same strong effect on biofilm bacteria as intracanal medicaments and in order to eliminate the chances of recurrent infection bacterial elimination is inevitable, however Peters reported that remaining microorganisms below the concentration of 10^2/ml at the time of canal filling has no negative effect on the treatment outcome (Peters et al. 2002). According to our study this concentration could be 100% reached by the application of CHX-gel alone or in combination with preparation and irrigation with NaOCl or CHX solution, placing in mind the aim of manual or mechanical root canal preparation besides eliminating the necrotic and dentinal debris is to remove the bacterial attachments to the canal wall and provide a wider lumen for the action of intracanal medicaments but never to fully eliminate bacterial colonization's since many anatomical irregularities, ramifications and accessory canals cannot be reached (Orstavik et al. 1991), the concentration also could be reached after $Ca(OH)_2$ application combined with chemomechanical preparation with NaOCl or CHX solution. The most effective treatment recommended according to our study is by the suggested intracanal medicaments with chemomechanical preparation with NaOCl or CHX solution. However we cannot give a clear recommendation to use the above treatments since our in vitro study is excluded from contamination factors and through the preparation and application of irrigants contamination may occur through oral microorganism or through the instruments themselves and this increases with the number of treatment sessions (Weiger et al. 2000), however our results are similar to those obtained by Ercan et al. whom investigated the effects of 2% CHX-gel on C.albicans and E.faecalis in comparision to $Ca(OH)_2$. Some studies state minimal effectiveness of $Ca(OH)_2$ against C.albicans (Waltimo et al. 1999, Ferguson et al. 2002), however these methods varied greatly in their examination procedure and probe preparation and also in the method of medicament application. In our study we did not place the medicament solution in direct contact with the medication outside the canal but followed the usual in vivo situation in which resistance occurs through adhesion and colonization of the dentinal surfaces and accessory canals, on the contrary to the clinical situation a higher amount on intracanal medicament is applied through the larger root canal lumen, however through sufficient amount of root filling this can be simulated. Still few studies are present about the canal disinfection with diode lasers. Gutknecht et al. 2000 and Moritz et al. 1997, could reach through the intracanal application of diode laser a reduction of streptococcus and staphylococcus to 10^3-10^4CFU/ml reached, in our study the unique laser utilization resulted in reduction to 10^6 CFU/ml which corresponds when converted to percentage to 99%. In vitro studies show many attempts to eliminate intracanal microorganisms especially E.faecalis which can reach up to 100% (Moritz et al. 1997, Gutknecht et al. 2000/ 2004, Schoop et al. 2006, de Souza et al. 2008) however the results obtained without previous preparation and medicaments are subjectively high. Sennhenn-Kirchner et al. investigated the effectivity of Er:YAG and 810 diode lasers against C.albicans biofilms attached to glass and titanium plates with 4x20s irradiations and 30s pause after each irradiation. The differences in irradiation periods, microbiological procedure and wavelengths used (810-980nm) make the direct comparison to our study invaluable. Chan et al. stated that different diode wavelengths can affgect their bactericidal activity, however this or presence of another bacterial species in the canal has a little scientific value since the bactericidal effect depends on the excessive heating and rupture of the bacterial cells and not on specific cellular elements. Even against heat resistant Bacillus stearothermophilus Nd:YAG laser irriadiation can be effective to achieve below 10^2 CFU/ml reduction (Hardee et al. 1994). However pigmentation affects bacterial sensitivity to great extent due to increasing the absorption coefficient , even for resistant bacteria a methylene blue pigmentation will increase their sensitiy to diode laser irradiation (Chan et al. 2003).

CONCLUSION:

CHX –gel and $Ca(OH)_2$ had perfect effectiveness againt C.albicans in root canals when combined with chemomechanical preparation and NaOCl and CHX irrigation , however laser disinfection showed less effectiveness and should be further investigated.

SPECIAL THANKS:

I would like to present my very grateful thanks to my dear colleagues dr. Nawar Ghazal and dr. Dimitris Papadimas at the Ruhr University of Bochum for making it possible to achieve this work, I really owe you more than I can say.

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The effects of polishing the fitting surface of acrylic base retainers on Methichillin resistance staphylococcus aureus; a laboratory study

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ABSTRACT
This *in vitro* study evaluated the effects of polishing the fitting surface of two acrylic based matetrials on the surface characteristics and biofilm formation of Methicillin Resistance *Staphylococcus aureus* (MRSA) biofilms. A simulated mouth model (Constant Depth Film Fermenter) was used to produce biofilms on autopolymerised and heat cure acrylic substrata. Surface parameters included surface roughness, hydrophobicity and surface free energy was evaluated after using the conventional polishing procedure to samples simulating the clinical situation. The results showed that MRSA has successfully adhered and grown in all samples with a similar pattern without a significant difference between the recovered viable bacterial counts. The rough surface provide protected niche for MRSA against the dislodgment forces. In conclusion, the finding of suggested that polishing the fitting surface of acrylic base materials may facilitate MRSA biofilm removal.

KEY WORDS
MRSA Biofilm, Hawley retainer, surface roughness

المستخلص
قيمت هذه الدراسة المختبريه آثار تلميع السطح المناسب لاثنين من مواد الأكريليك على أساس الخصائص السطحية وتشكيل بيوفيلم (الأغشية الحيوية) لجرثومة المكورات العنقودية الذهبية مقاومة المثسلينز تم استخدام محاكاة نموذج الفم (مخمر الاغشيه ذوالعمق التابت) لإنتاج الأغشية الحيوية على مادتي الاكريليك تلقائي التصلب و الاكريليك الحراري شملت المعلمات خشونة السطح، الخاصيه للا مائية و الطاقة الحرة للسطح وتم تقييمها بعد استخدام إجراء التلميع التقليدية للعينات المحاكايه للحالة السريرية. أظهرت النتائج أن هذه الجرثومة نمت في جميع العينات مع وجود نمط مماثل دون اختلاف كبير بين أعداد البكتيريا. لقد وفر سطح خشن حماية ملائمة لهذه الجرثومة ضد قوات الخلع.
في الختام، اقترح العثور على اسلوب مناسب لتلميع اسطح مواد الاكريليك بما بسهل عمليه إزالة بيوفيلم هذه الجرثومة.

INTRODUCTION:
The oral cavity is one of the most heavily populated regions of the human body with regards to microorganisms. Various factors may influence the diversity of the oral microorganisms in an individual such as age, sex, genetics and diet [1]. However, it is difficult to define the composition of the oral microbiota precisely because of the frequent exposure to the exogenous microorganisms in water, food, air or during direct contact to other microbial communities. It has been suggested that the lack of adhesins and receptors are among the causes that discourage the exogenous microorganisms to bind or co-aggregate to the oral surfaces [2].

It is not always true that the oral microbiota exhibit a beneficial effect on the host. Indeed, there is a dynamic association between humans and their microbiota [3]. The characteristic composition of the commensal oral microbiota can be disturbed by altering the habitat which affects the stability of the oral environment, such as pH, the supply of oxygen and the relationship between the communities. These changes can lead to various oral diseases such as dental caries, gingivitis and periodontal disease [4, 5]. Moreover, the oral cavity may act as a reservoir for infection at remote sites such as bacterial endocarditis or brain and liver abscesses when gaining access to the bloodstream via untreated carious lesions or the gingival crevice [6-8]. Indeed, it has been suggested that oral microorganisms may be diagnostic biomarkers for some diseases such as in pancreatic disease [9, 10].

Biomaterials are prone to microbial accumulation during and after implantation. The microorganisms adhere to these materials by either specific interaction or non-specific interactions [11, 12]. Acrylic resins are widely used for the construction of various dental prosthesis including orthodontic appliances. These materials are prone to microbial adhesion and biofilm formation [13]. Staphylococci and MRSA have the ability to adhere to acrylic base retainers and form biofilms [14]. Acrylic materials may act a reservoir for these opportunistic microorganisms.

The aim of this study was to find out whether polishing the fitting surface of acrylic base removable orthodontic retainer materials influences surface properties that discourage MRSA biofilm formation.

MATERIALS AND METHODS
Three moulds of silicone (irreversible silicone duplicating material, Dentaurum, Germany) were made from a smooth glass block 100 mm x 80 mm of known roughness value. A class IV stone (Crystacal

R, BPB Formula, UK) was vacuum mixed according to the manufacturer's instructions and poured to produce a positive replica for the reference block.

An acrylic sheets of 1.5 mm thickness of autopolymerised acrylic (Forestacryl, Forestadent, Germany) and heat cure acrylic resins (Meadway, Bracon Ltd., UK) were constructed, using the addition technique and the compression moulding technique respectively, according to the manufacturers' instructions [15].

The acrylic sheets were then divided into two halves; one was finished using a conventional laboratory technique with a tungsten bur (Bracon) at 15000 rpm followed by polishing with a slurry of pumice (Pumice, Dentsply, UK), water, bristle brush including lathe and Calico Mop (C&L.E. Attenborough Ltd., Nottingham, UK) and polishing composition (Vonax, Canning-Lippert Ltd., Birmingham, UK). The surface of the other half was kept without modification to simulate the clinical condition.

Five samples of 15 mm x 10 mm of each material described above were tested with regard to their hydrophobicity and surface free energy. The contact angle of distilled water (for hydrophobicity), glycerol (Sigma-Aldrich) and hexadecane (Sigma) were performed according to a sessile drop method [16].

For measuring the surface roughness, five samples of 5 mm in diameter of heat cured and autopolymerised acrylic were analysed using atomic force microscope (AFM; XE 100 Park Instruments Korea) to measure the surface roughness value Ra (arithmetic roughness). The roughness value represents an area of 45 x 45 µm and consists of 512 x 512 pixels with a scan scale of 1 Hz. The probe has a bending constant of 0.3 N/m.

In vitro studies were performed to assess the effects of different retainer materials and their surface properties on biofilm growth. For this purpose the Constant Depth Film Fermenter (John Parry Jones Engineering, Cardiff, UK) was used as described previously [17].

The biofilm was quantified and viewed using the same protocols as described by Morgan and Wilson [18].

The Results obtained from the biofilm assay were tested, after logarithmic transformation of the data, using independent student's t-test to compare the differences in the colony forming unit means.

RESULTS

Table 1 shows the surface physiochemical properties including surface roughness, hydrophobicity and surface free energy of the acrylic and thermoplastic samples. The surface roughness values of the autopolymerised acrylic samples were higher than that of the heat cure samples (0.93µm and 0.66µm respectively). However, the polished samples were almost similar. It has also found that when the surface roughness increases the contact angle increase. Hence, the material became more hydrophobic. Simultaneously, the samples showed no difference in total free energy and Van der Waals forces.

The deposition of MRSA on heat cured and autopolymerised acrylic samples mimicking the clinical situation and those finished and polished using laboratory polishing procedures are shown in figures 1 and 2. There were no significant differences in the recovered MRSA counts between the polished and unpolished acrylic samples on either of the resin materials although the bacterial counts recovered from the polished surfaces were less than that of the unpolished ones.

However, visually the SEM and the CLSM micrographs (figure 3) show that the pattern of MRSA aggregation on the clinically simulated samples was different from that occurring on the conventionally polished ones. The later showed that the bacteria adhered initially in the rough irregularities remaining following the conventional polishing procedure, leaving the polished area with very few numbers of bacteria. The same pattern of bacterial distribution was detected on the clinically simulated samples where the MRSA aggregate was denser in the deeper irregularities within the rough areas of the surface, however, the bacteria still aggregated on the rest of the sample.

Table 1: Surface roughness (Ra in µm), hydrophobicity (in degrees) and surface free energy parameters (in mJ/m2) of heat cure acrylic and autopolymerised acrylic samples polished using the conventional polishing procedure. The surface free energy parameters include Lifshitz-Van der Walls (LW), electron donor (-), electron acceptor (+), acid-base component (AB) and total free energy (Total).

Materials	Surface roughness	Hydrophobicity	Surface Free Energy				
			LW	(+)	(-)	AB	Total
Cold cure acrylic unpolished	0.93±0.45	75.7±4.5	21.8	0.005	18.21	0.6	22.4
Cold cure acrylic polished	0.27±0.19	72.1±5.9	19.44	0.16	20.96	3.66	23.1
Heat cure acrylic unpolished	0.66±0.23	81.1±3.1	21.24	0.01	14.72	0.77	22.01
Heat cure acrylic polished	0.25±0.1	75.9±6.3	21.9	0.01	25.05	1	22.9

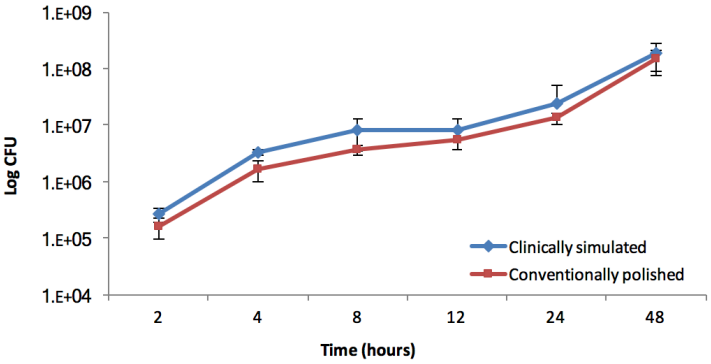

Figure 1: Viable counts of MRSA deposition on clinically simulated autopolymerised acrylic samples versus conventional (laboratory) finished and polished samples.

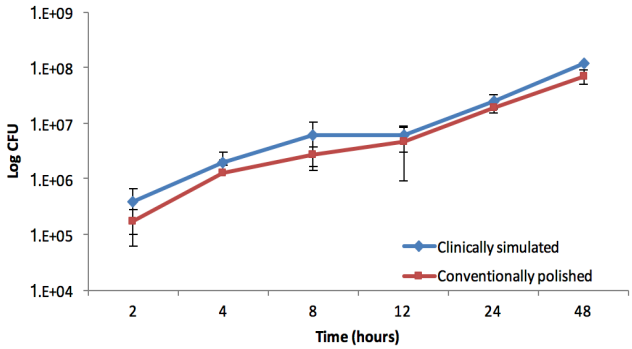

Figure 2: Viable counts of MRSA deposition on clinically simulated heat-cured acrylic samples and conventionally (laboratory) finished and polished samples.

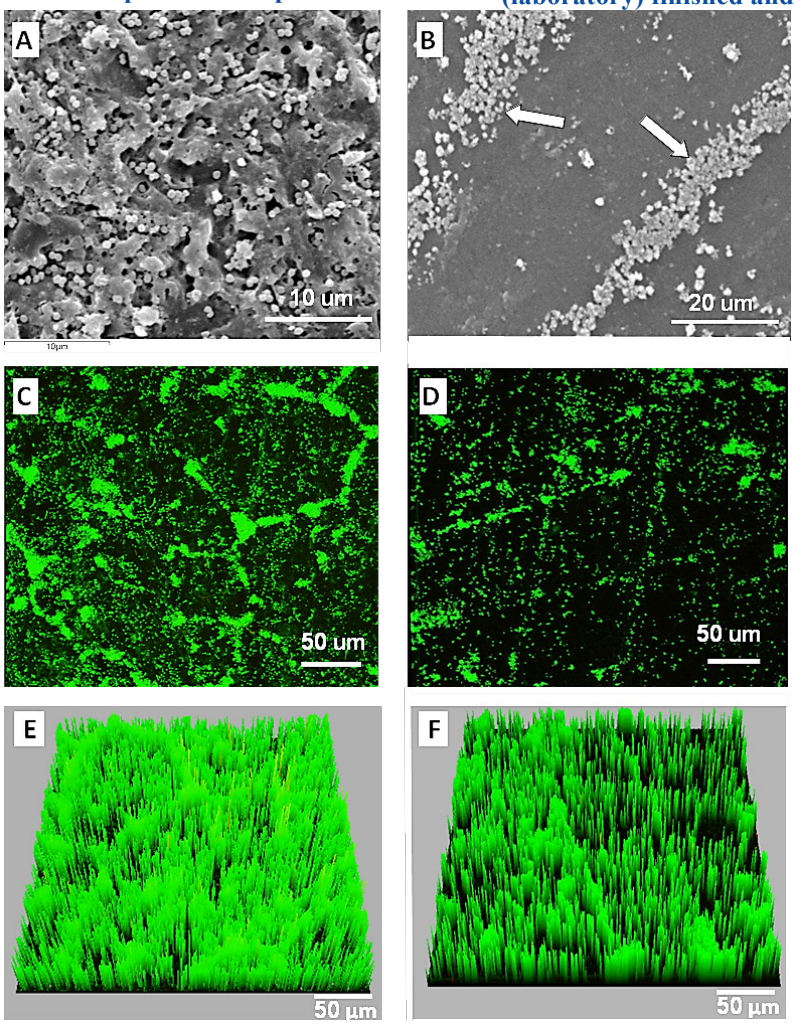

Figure 3: SEM and CLSM images with three dimensional representation of 24h MRSA deposition on heat cured acrylic samples receiving surface modification. Unpolished heat cured acrylic (simulate clinical condition) (A, C and E); Polished heat cured acrylic sample using conventional polishing procedures (B, D and F). The arrows refer to the distribution of bacterial attachment.

DISCUSSION

Removable orthodontic retainers are intraoral implants and are subjected to the same problems as other implants in that they are susceptible to biofilm accumulation. In a previous study MRSA was recovered from the retainers and the oral cavity of full time retainer wearers [14]. It is well known that surface roughness increases the physical surface area of a material and may provide protected niches where the bacteria are sheltered against the dislodgement forces such as mechanical brushing. A threshold surface roughness value for microbial aggregation of 0.2 µm has been suggested by some *in vivo* studies in that below this value, no further reduction in microbial accumulation could be detected [19].

To ensure clinical relevance, the biofilm assay was undertaken using materials that are already applied in orthodontics; therefore, the substratum was fabricated

following the manufacturer's recommendations and finished using the same laboratory procedure. Furthermore, a CDFF was used which generates large numbers of reproducible biofilms with conditions similar to that of the oral cavity [20].

Several studies have been conducted to find the effect of surface roughness of acrylic materials on *Streptococcus* spp. or *Candida* spp. attachment [18, 21]. However, studies involving staphylococci biofilms have only been carried out using bone cement as a substratum [22].

The results obtained from the current study revealed that all the tested samples showed hydrophilic surfaces and when the surface became rough, the contact angle increased and the surface exhibited more hydrophobic characteristics. This may be due to the barrier effect of the surface irregularities which may prevent the spread of the water drop. This comes in agreement with Crick and Parkin [23] who found that the wetting characteristic changes when a surface becomes rougher.

Although the surface free energy is influenced by the chemical composition of a substratum and the fabrication procedure which in turn affects the surface properties of that material [24], the current study showed that there were no marked differences in the total surface free energy and Van der Walls force regardless of difference in surface roughness.

The biofilm data showed that MRSA successfully adhered and colonized forming biofilms on the surface of acrylic substrata constructed simulating the clinical condition. When the surface roughness of a material increased the bacterial count increased regardless of the type of tested material; however, there was no significant difference between the clinically simulated and the laboratory polished samples. The result of this study showed that MRSA was detected within the microscopic surface irregularities of the unpolished samples and accumulated initially in the rough areas within the polished acrylic samples. The surface free energy parameters did not have an obvious effect on bacterial attachment. Surface irregularities in rough material increase the physical surface area and provide protected niches that encourage bacterial adhesion [25].

CONCLUSION

The data obtained from the current study showed that acrylic materials have shown favourable surface characteristics for MRSA adhesion and biofilm formation. Surface roughness may influence MRSA biofilm formation on acrylic. Smooth surfaces may facilitate biofilm removal, whereas a rough surface not only increases the physical surface area but also provides shelter for the adhered bacteria against mechanical dislodging forces and, therefore, anchor the established biofilm.

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Effect of Different Surface Treatments on The Tensile Bond Strength Between Acrylic Resin Teeth and Denture Base Material

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ABSTRACT

Aims of study: A study was carried out to assess the tensile bond strength between acrylic denture base and denture teeth after manipulation with different surface treatments. **Methods**: Thirty central incisors from acrylic denture teeth were cut at the neck (ridge lap surface). Such teeth were then allocated into three groups of different surface treatments: teeth in the first group received no further treatment; teeth in the second group were surface treated with a groove placed and reinforced with a metal wire; teeth in the third group were reinforced with a glass fiber. Each group was processed by water – bath. **Results:** The results showed that all treated groups improved the tensile bond strength was in a significant, level at (P<0.01), while the third group glass fiber was the highest mean values. **Conclusions:** The metal wire enhanced significantly the tensile bond strength. The addition of glass fiber can significantly enhance the tensile bond strength between acrylic denture base and denture teeth.

KEY WORDS

Acrylic resin, Acrylic teeth , glass fiber, Tensile strength.

المستخلص

ان الهدف من هذهَ الدراسة هو تقييم قوة الشد بين قاعدة الطقم الاكريلي والاسنان بعد معالجة السطح.

تم اختيار ثلاثون نموذج من الاسنان (القواطع) بعد عمل عمل خدود في منطقة العنق ثم قُسمت الى ثلاثة مجاميع:

المجموعة الأولى: عشرة الاسنان (القواطع) لم تتعرض الأسنان لمعالجات إضافية.

المجموعة الثانية: عشرة الاسنان (القواطع) تم عمل اخدود فيها وثم اضيف واير معدني.

المجموعة الثالثة: عشرة الاسنان (القواطع) تم اضافة فيها مادة الالياف الزجاجية.

تم طبخ جميع العينات حراريا َبواسطة جهاز الحمام المائي.

أظهرت النتائج ان جميع المجاميع التي أدخلت عليها المعالجة قد حسنت من قوة الشد الا ان المجموعة الثالثة قد أظهرت قوة الشد عالية جدا.

نستنتج بان معالجة السطح بواسطة إضافة واير معدني اظهر تحسنا في قوة الشد اما الالياف الزجاجية فقد أظهرت تحسنا عاليا في قوة الشد.

INTRODUCTION

Acrylic resins are the most commonly restorative materials used to make removable complete and partial dentures. Such materials to be polymerized require the activation of an initiator, such as benzoyl peroxide, to free radicals, in an addition polymer. The heat is usually generated either in a hot water bath or by micro waves [1]. The most commonly method used to process poly (methyl methacrylate) resin is the water bath [2, 3].

Plastic denture teeth are often preferred over porcelain teeth as they are easier to adjust and chemically bond to the denture base materials. Heat-cured acrylic resins are the materials of choice for denture bases [4,5]. The failure rate of acrylic resin dentures due to the fracture has been reported to be an acceptable high [6]. The most common type of failure encountered was de-bonding or fracture of the teeth [7]. The lack of adequate bonding at the tooth - base interface was due to:

a. Contamination of the surface, particularly by wax and possibly by sodium alginate mold seal.
b. The difference in the structure of the components due to their different processing routes [8].

The failure of the bond between acrylic resin teeth and denture base material remains a significant problem. The literature indicated that the chemical and mechanical surface treatments of the denture tooth surface prior to bonding have enhanced the bond strength [9]. The current study was aimed to evaluate the effect of different surface treatments (reinforced metal and glass fiber) on the tensile bond strength between acrylic teeth and acrylic resin denture base material.

METHODS:

Grouping of the specimens:

Thirty teeth from acrylic resin were selected and all located into three different surface treatment groups as follow:

1. First group (10 samples): the teeth did not receive any treatment.
2. Second group (10 samples): the teeth were surface treated with a groove preparation and reinforced with a metal wire (Pigeon dental, Dentirak) .
3. Third group (10 samples): the teeth were surface treated with monomer and reinforced with glass fiber (Vetrotex –ocv reinforced, mouldingLtd.,UK) .

Preparation of specimens:

Preparations of teeth:

Thirty central incisors were selected and were cut at the neck (gingival portion) as follow:

A rubber mold-casting ring was used to prepare (round form) stone bases. According to the manufactures instructions, dental stone was mixed and poured in the rubber mold. Approximately 4mm of the incisor portion of each central incisor was embedded in the stone mixture in such way that the gingival

portion of the tooth portion of the tooth parallel to the horizontal plane [10]. The stone bases with teeth were fixed on surveyor table by the screw. A grasping unit was designed and prepared for this study for holding a portable engine hand piece in a fixed position (parallel to the vertical arm and perpendicular to the surveyor table) and allowed horizontal movement of the hand piece with surveyor arm. The surveyor arm allowed also a vertical movement of the hand piece. By this a groove was placed on the tooth neck surface. A portable engine hand piece waxed fixed on the surveyor arm, and with constant speed (4000rpm) was used for cutting by using at a stone disk bur for each tooth and in one-way direction to the ridge lap portion of each tooth was cut.

Mold preparation:

A wax mould made form rectangular shape, dimensions of (17mm,10mm,7mm,9mm,3mm) [5] as shown in the figure (1).

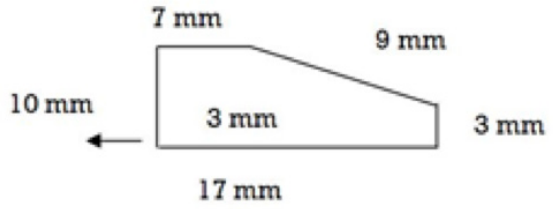


Figure (1): Wax mould for a rectangular wax block.

The teeth were waxed on the beveled surface of a rectangular wax block. The slope of the beveled surface aligned each denture tooth so that the long axis of the tooth was at (45) degree from the base of the wax block, figures (2 and 3).

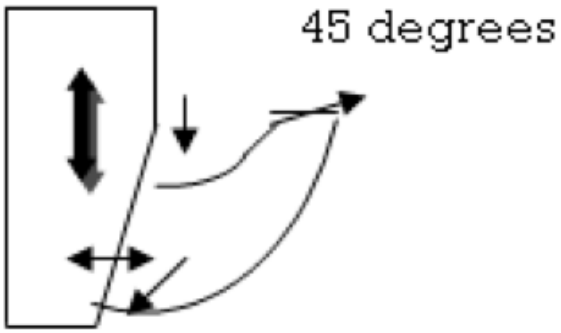


Figure (2): Slope of the beveled surface aligned each denture tooth.

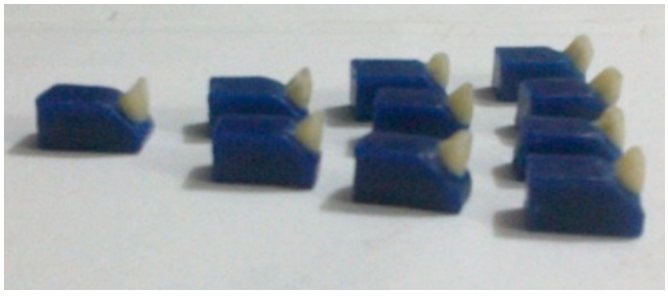


Figure (3): Specimens (after fixed each tooth).

Addition of fiberglass

Electronic balance was utilized to weigh the amount of fibers that used in this study. Short fibers (0.375 grams) were added randomly to the acrylic powder and mixed together. The acrylic monomer was then added to the powder and mixed using cement spatula. When the mixture reached a dough stage. The acrylic was packed within the stone mould, cured and finished [11].

Addition of reinforced metal wire

Ten acrylic maxillary central incisors were selected and cut at the neck (gingival portion) using fissure bur for each tooth and in one-way direction to the ridge lap portion. Next, a stainless steel wire of (9 mm) diameter and (6 mm) length was selected. A half of wire length was fixed in tooth groove via adhesive material and the rest was embedded on wax pattern [12].

The conventional flasking technique for complete denture was followed in the mold preparation as previously described. The lower half of the flask was completely filled with dental stone, which mixed according to manufacture instruction (100gm/31mL), (p/w). The wax patterns were then inserted to one half of its depth (figure 4). The stone and wax pattern were lubricated with separating medium and allowed to dry. The upper half of the flask was placed over the lower half and then filled with stone. The mould was left to set for (60 min.) before mixing to the next step.

Figure (4): The wax pattern when insert in stone.

Pink heat cure acrylic powder with liquid was mixed according to manufactures instruction (3gm/1ml) (p/L). The teeth were properly positioned within the mould. When the mixture reached a dough stage, the acrylic dough was then placed inside the mould. The two halves of the flask were finally closed under pressure until metal to metal contact had been established and left under press (20 bar) for (5 min.)[13]. The curing was done in a thermostatically controlled water bath (temperature at (74℃) for (2½ hour) then in (100°C) for ½hour [14]. Heat source was then switch on and left for (90 min.) and then boiled for (30 min.). After that, the flask was allowed to cool down in the water bath [15]. Once curing, the specimens were

removed carefully from the stone mould. The samples were then finished and polished [16] figure (5). Before testing, all specimens were stored in distilled water (37ºC) in 10 days [5].

Figure (5): specimens after finishing and polishing

Tensile Bond Strength Test:

All specimens were tested in the same day. The specimens were hold in metal fixture (grasping unit). The metal fixture was firmly hold to an instron universal testing machine model 1195 (Instron, corporation, canton mass). The compressive load was applied with a loading testing machine at (45) degrees from the long axis of each denture tooth on the palatal surface (figure 6). Tensile bond strength test was then performed using a (200 kg) load cell with a cross head speed 0.5 mm/min with a chart speed (20 mm/min.). Specimens were loaded until fracture. The data was recorded in kilograms. The tensile bond strength was calculated according to the formula below: -

$$T.S=\frac{F}{S}$$

$$S=\frac{\pi 2}{4}XD$$

where $\pi = \frac{22}{7}$

D (diameter) =5mm, S = 19.64 mm^2

TS = tensile strength (N/mm^2)

F= Force at failure (N).

S= area of cross section [17].

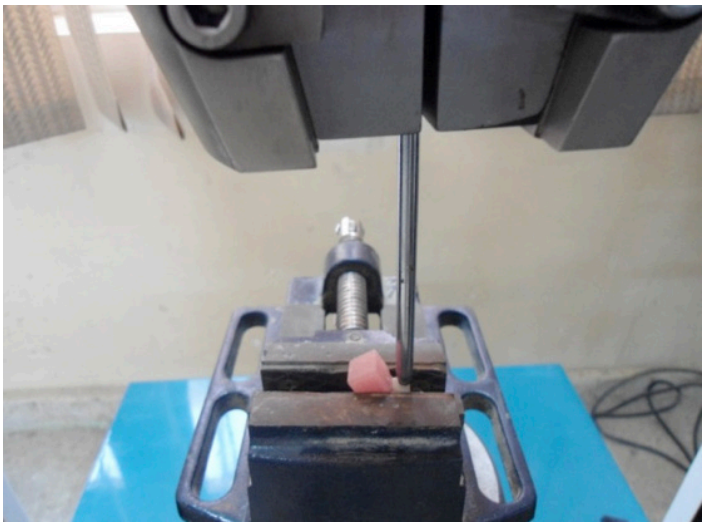


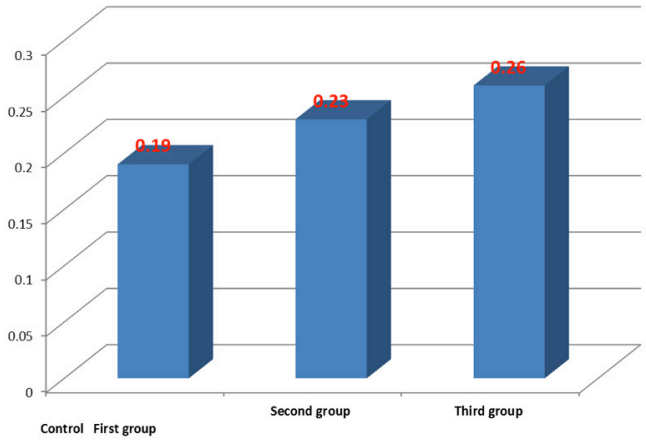
Figure (6): specimen with chucks in place ready for test.

RESULTS

All values of tensile bond strength were shown in the Table (1).

The table (1) and figure (7) showed that the specimens which were not surface treated had a low tensile bond strength (0.19 N/mm^2). Furthermore, the specimens which treated with reinforced metal wire had a mean value (0.23 N/mm^2).

Moreover, the specimens reinforced with glass fiber were the highest tensile bond strength value (0.26N/mm^2).



Figure(7): Disgram showing the distribution oftensile bond strength among studies groups.

Table (1): Descriptive statistics of mean distribution of tensile bond strength among studies groups.

Studies groups	*Mean(N/mm^2)*	*Standard deviation*	*Stander error*	*Minimum*	*Maximum*
First group (Control)	0.19	0.021	0.006	0.16	0.22
Second group	0.23	0.0205	0.0065	0.21	0.26
Third group	0.26	0.0188	0.0059	0.24	0.29

The results demonstrated that there were statistically significant differences between the studied groups as shown in tables 1, 2, and 3. The ANOVA test indicated that there were highly

significant differences between all groups as shown in table 2.

Table (2): ANOVA test between groups tensile bond strength among studies groups.

	F-test	P-value	Sig
Between groups	29.73	P<0.01	HS

Table (3): Least significant difference (LSD) test for tensile bond strength among studies groups

(LSD) test			
Studies groups	First group	Second group	Third group
First group	-	HS	HS
Second group		-	- S
Third group			-

DISCUSSION

Many studies have been carried out to assess and improve the bond strength of acrylic teeth to denture base material. Bond strength can be affected by several factors, including cross linking of the materials, availability of the monomer, and the degree of contamination during processing. Several procedures for repairing acrylic dentures are employed by Japanese dental laboratories, to repair using wrought wire. The denture is prepared by cutting groove at the broken area. The groove should made slightly wider than the wrought wire and deep enough so that the wire can completely seated in the groove and fixed in it. The joint design is important in this process to success of the repair [18 ,19].

In this study, the bond strength between acrylic resin teeth and denture base resins were evaluated in tension. Results showed that the most of tensile bond strengths are a mostly around or less than that determined by denture teeth. This is best explained by the differences in the experimental design. The result of this study showed the tensile bond strength of acrylic teeth, as a function of different reinforcement to denture base resin was significantly at (P value<0.05). This result is in agreement with Takahashi et al. and others [5, 20&21]. While our results disagree with Geert & Jooste and others [22, 23& 24]. A possible explanation for such differences in the results is the difference in the experimental design. The previous studies applied compression load at (45) degrees to the palatal surface of a denture tooth mounted on a rectangular resin block (thickness was not mentioned). In this study teeth after cutting were allocated into three groups previously mentioned, the first group (control) were the teeth were not surface treated, in the 2nd group the teeth were treated with groove and reinforced with a metal wire, in the 3rdgroup the teeth were conditioned with monomer with addition of reinforced glass fiber. The results showed that all surface treatments had significant difference at (p_{value}<0.05), except the control are non-significant difference at (P_{value}>0.05) in improving tensile bond strength mean value as shown in table (2, 3, and 4). This is agreement with Takahashi et al., and others [5,21, &25].

The results of the current study revealed that there were significant differences among the tested groups. The groups reinforced with a metal wire has a higher mean value of tensile bond strength than untreated group. This may be due to adding of reinforced metal wire give support to the bond between acrylic teeth and acrylic resin denture base and give it higher tendency and stability to withstand a higher tensile bond strength. This study agreed with Polyzios et al., [12] and Suzuki [26].

In addition, the samples that were surface treated by addition of glass fiber had higher mean values of tensile bond strength when compared with control group. This can be partly explained by the presence of glass fiber in resin ensures transferring of load from matrix to fiber. This will lead to an increase in the strength of the resin and allows the resin tolerate the force of tension more than the samples that had no fiber in their structure. Adequate quantity of fiber present in resin can increase the acrylic strength. This is in agreement with Ehasn [11], and disagree with Polyzios et al., [12].

CONCLUSIONS:

The following conclusions can be drawn:

1. A higher bond strength of acrylic denture base to the acrylic teeth was obtained with metal wire as compared to untreated group.
2. The addition of glass fiber can enhance the tensile bond strength.

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The Effect Of Meloxicam and Mefenamic Acid Premedication on Pain Experience In Orthodontic Patients

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ABSTRACT
Background: One of the side effects of orthodontic treatment is pain this lead to discourage patients from treatment. The aim of this study to estimate and compare the effect of preoperative use of Meloxicam and Mefenamic acid drug on pain experienced after separator placement in orthodontic patients.
Materials and Methods: Thirty patients aged between 17 and 26 years who claimed to undergo orthodontic treatment with fixed appliance were participate in this double-blind, prospective study. They were randomly distributed into three experimental groups, 10 for each group as follow: group A; administration of starch capsules, group B; administration of 500 mg mefenamic acid, and group C; administration of 7.5 mg meloxicam; medications were administered 1 hour before separator placement. The pain was recorded by the patients on a linear and graded Visual Analogue Scale (VAS) at time intervals of 2 hours, 6 hours, nighttime on the day of appointment, 24 hours after the appointment, and 48 hours after the appointment during each of these four activities: (chewing, biting, fitting front teeth, and fitting back teeth).
Result: The results of ANOVA reveal high significant differences between the three experimental groups at all time intervals and during all four activities ($P < .05$). LSD comparisons reveal high significant differences between placebo, mefenamic acid, and meloxicam groups ($P < .05$) the lowest pain was reported by the meloxicam group, and the highest one in placebo group.
Conclusion:
- The use of 7.5 mg meloxicam tab or drug as a single dose 1 hour preoperatively only is highly recommended for pain control after separator placement.
-The pain begins at the moment of separator placement and reaches its peak in 24 hours and then, gradually decreases until the 48 hours.

KEY WORD
Orthodontic pain. Mefenamic acid. Meloxicam

INTRODUCTION
Approximately 90% of the Orthodontic patients report pain [1], making it the most commonly reported detrimental effect of Orthodontic treatment and the greatest reason for wanting to discontinue or avoid orthodontic care [2.3]

Pain is caused by some tissue changes. These tissue changes in orthodontic treatment are caused by the compression of periodontal ligament and alteration of blood flow to the tooth, resulting in releasing chemical mediators like prostaglandins [4, 5, 6].

It has been claimed that degree of pain experienced by patient varies based on gender, age, patient anxiety level, and emotional stress [1, 6, 7, 8]. When compared with the pain associated with extractions, both the incidence and severity of orthodontic pain is perceived to be greater [6, 9]

The conventional nonsteroidal anti-inflammatory drugs (NSAIDs) like Mefanamic acid, ibuprofen, piroxicam, aspirin and naproxen sodium produce their analgesic, antipyretic and anti-inflammatory effect by blocking the production of prostaglandins through inhibiting the other isoforms of cyclooxygenase (COX) enzyme [10] these medications are called non-selective COX inhibitors, since they block both COX_1 and COX_2 isoforms [11].

Meloxicam is one of most popular relatively selective COX_2 inhibitors used in the treatment of acute and chronic inflammatory painful disorders like rheumatoid arthritis, dental pain, and postoperative pain [12-14]. It is now clear that meloxicam has a lower gastric effect compared to other NSAIDs [13]. Efficacy of this drug in controlling post-endodontic pain and pain after third molar removal and oral surgery has been investigated previously [13.14.15].

The aim of this study is to estimate and compare the effect of preoperative use of Meloxicam and Mefenamic acid on pain experienced after separator placement in orthodontic patients.

We think this kind of information is necessary in clinical practice, enabling the health care professionals to consider all the factors related to the orthodontic treatment, and to decide on the best individual therapeutic plan for each case.

MATERIALS AND METHODS:
The study was conducted in Baghdad. The sample comprised of 30 patients (15 males and 15 females) who were scheduled to receive fixed

orthodontic treatment agreed to be part of this study. The selection criteria were: the patient must be (1) not younger than 15 years and not older than 30 years of age; (2) never have any previous orthodontic treatment; (3) have no contraindications or adverse effects related to mefenamic acid and meloxicam; (4) not using any antibiotics or other analgesics during conducting the study; and (5) have a minimum weight requirement of 40 kilograms, as per Food and Drug Administration– approved over-the-counter pediatric dosage labeling guidelines.

Ten patients were evenly and randomly distributed to the three experimental groups as follow; group A (starch capsule), group B (500mg mefenamic acid), and group C (7.5mg meloxicam). For all groups, medications were administrated one hour prior to separator placement as one tablet.

A 100-mm horizontal visual analogue scale (VAS) was selected to measure the degree of discomfort [17] and was given to the patients in the form of a 6 pages booklet with possible answers of "no pain" (0 mm) and "worst pain" (100 mm). The patients were instructed to mark the degree of pain/discomfort at the appropriate time intervals by placing a mark on the scale and indicating the severity of pain/discomfort during four different activities: biting, chewing, fitting back teeth together, and fitting front teeth together. These were recorded by the patients at the following intervals: 2 hours post treatment; 6 hours post treatment; bedtime/nighttime on the day of the appointment; 24 hours after the appointment; and 48 hours after the appointment.

Statistical analysis

All Statistical analyses were done using the Statistical Package for Social Sciences (Version 22.0, SPSS Inc., Chicago, Illinois, USA). Descriptive analyses were performed for pain scores for the experimental groups at each time intervals. Comparison between the three experimental groups in the four activities was made using one way analysis of variance (ANOVA). If the results of ANOVA were significant, least significant difference (LSD) test was used to find any statistical significant difference between each two groups. The level of significance for all tests in our study was set at $p < 0.05$.

RESULTS

From this study; descriptive statistics for the experimental groups are given in table 1. The mean age of the subjects in the three experimental groups was 22.14, and there was no significant difference between their mean ages ($P < .5$).

Table 1: Experimental Groups with Preoperative Analgesic, Mean Age, and Sex Distribution

Group	*Preoperative medication*	*Drug dose*	*Mean age*	*Std. Deviation*	*Gender Male/Female*
A	placebo	1 Capsule	22.13	3.22	5/5
B	Mefenamic acid	500 mg	21.29	1.59	5/5
C	Meloxicam	7.5 mg	23.00	2.00	5/5

Differences in Pain score between experimental Groups in "Pain on biting":

The results of ANOVA reveal highly significant differences ($P < 0.001$) among the placebo, mefenamic acid and meloxicam groups and a significant different in the time interval ,2 hours post treatment; 6 hours post treatment; bedtime/nighttime on the day of the appointment; 24 hours after the appointment; and 48 hours after the appointment.

Patients who were administered meloxicam experienced less "pain on biting compared with patients in the placebo group ($P < 0.001$) (Figure 1; Table 2).

Table 2: The mean, standard deviation and p value of pain level in different conditions

Biting											
Experimental Groups	*2h mean*	*SD*	*6h mean*	*SD*	*At night mean*	*SD*	*24h mean*	*SD*	*48h mean*	*SD*	*P value*
Group A: placebo	63.8	4.21	67.7	3.13	73.3	3.65	76.5	3.98	65.6	4.65	
Group B: mefenamic acid	32.9	2.85	54.3	3.40	60.5	3.37	70.2	3.74	55.0	2.98	0.000*
Group C: meloxicam	15.5	1.90	43.0	3.80	49.9	4.56	51.5	2.64	39.0	4.22	
P value	0.000*										
Chewing											
Experimental Groups	*2h mean*	*SD*	*6h mean*	*SD*	*At night mean*	*SD*	*24h mean*	*SD*	*48h mean*	*SD*	*P value*
Group A: placebo	41.5	4.12	57.20	3.46	62.4	4.27	66.7	3.83	60.5	3.03	
Group B: mefenamic acid	30.4	3.95	46.2	3.52	57.2	3.61	54.6	3.60	49.7	3.65	0.000*
Group C: meloxicam	11.6	2.41	17.5	2.42	27.0	2.98	30.6	2.67	22.6	3.17	
P value	0.000 *										
Fitting the front teeth											
Experimental Groups	*2h mean*	*SD*	*6h mean*	*SD*	*At night mean*	*SD*	*24h mean*	*SD*	*48h mean*	*SD*	*P value*
Group A: placebo	48.4	5.13	68.5	4.74	75.4	3.95	76.4	5.89	72.4	5.72	
Group B: mefenamic acid	30.0	3.50	50.1	3.21	66.4	2.41	72.1	2.33	61.0	3.33	0.000*
Group C: meloxicam	13.7	3.06	20.2	2.90	38.9	4.15	42.9	4.75	38.2	4.76	
P value	0.000 *										
Fitting the back teeth											
Experimental Groups	*2h mean*	*SD*	*6h mean*	*SD*	*At night mean*	*SD*	*24h mean*	*SD*	*48h mean*	*SD*	*P value*
Group A: placebo	44.0	7.38	62.2	6.48	63.2	6.70	62.4	6.19	47.7	4.45	
Group B: mefenamic acid	32.1	2.33	32.2	2.53	44.7	2.79	58.4	2.72	44.2	2.10	0.000*
Group C: meloxicam	10.1	4.25	12.2	2.53	19.4	3.13	23.5	2.42	24.1	3.41	
P value	0.000 *										

***Highly significance (P < 0.001)**

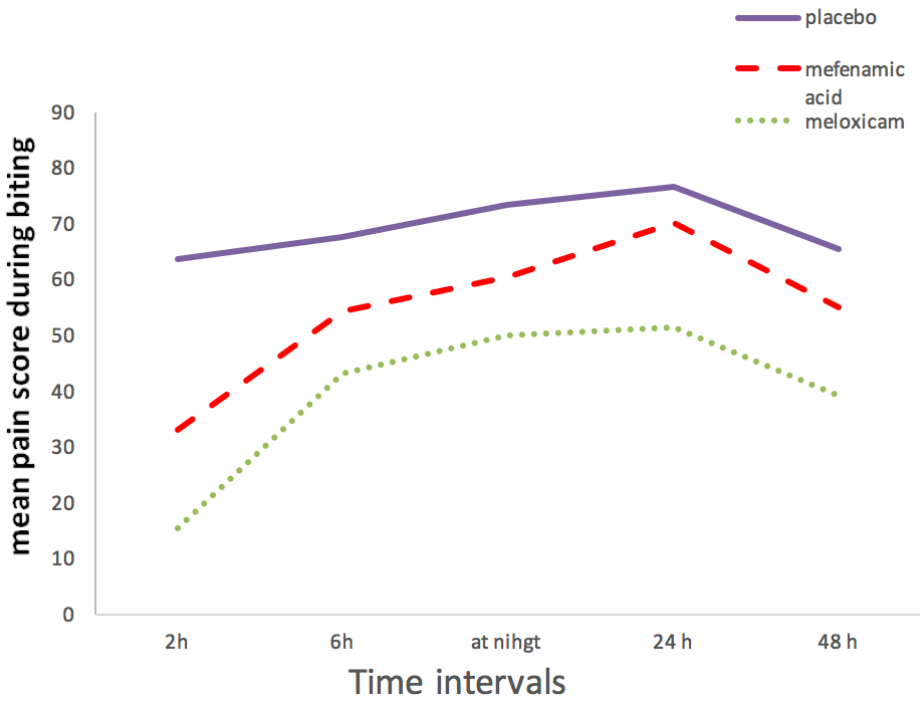

Fig. 1: Comparison of the mean pain scores on VAS among the three study groups over time on biting

Differences in pain score between experimental groups in "Pain on Chewing":

From Table 2; highly significant differences were shown among the placebo, mefenamic acid and meloxicam groups ($P < 0.001$) (Figure 2; Table 2). The pain scores at 2 hours, 6 hours, nighttime, 24 hours, and 48 hours after the separator placement appointment showed the meloxicam group to be the lowest among other experimental groups. This study showed the mean pain scores on chewing were less than on biting and more than on fitting front and back teeth.

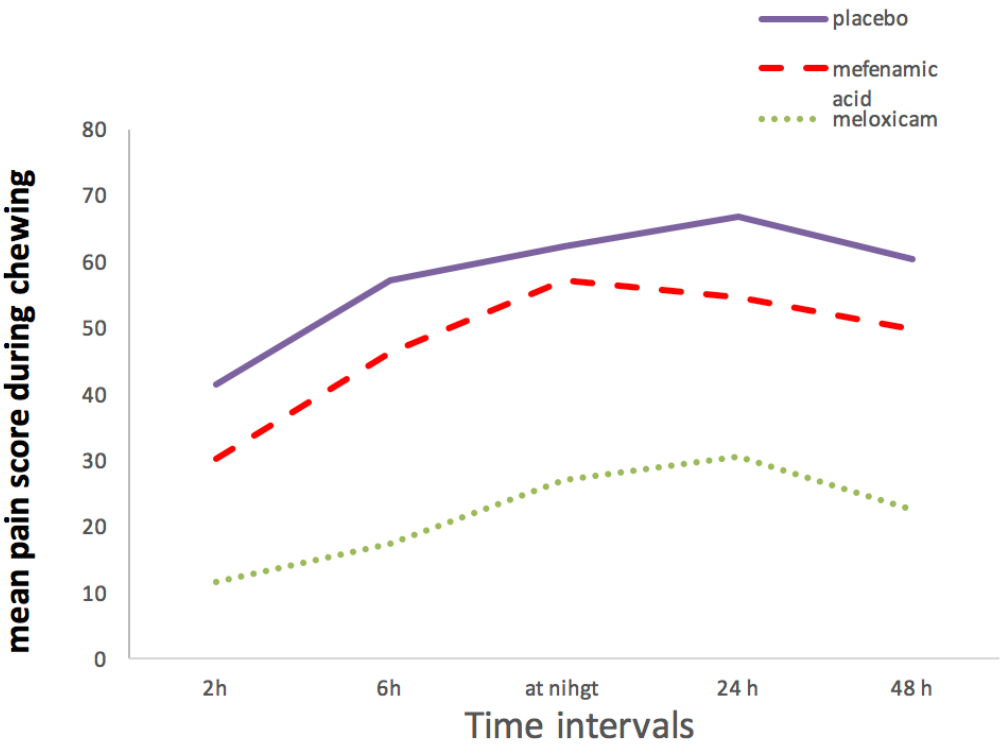

Fig. 2: Comparison of the mean pain scores on VAS among the three study groups over time on chewing

Differences in Pain score between experimental groups in "Pain on Fitting the front teeth"

With respect to pain level on fitting anterior teeth together, patients administered meloxicam showed highly significant ($P < 0.001$) less pain scores than mefenamic acid and placebo groups at all different time intervals.

This study showed the pain scores on fitting the front teeth was less than on during biting or chewing and more than on fitting posterior teeth (Figure 3; Table 2).

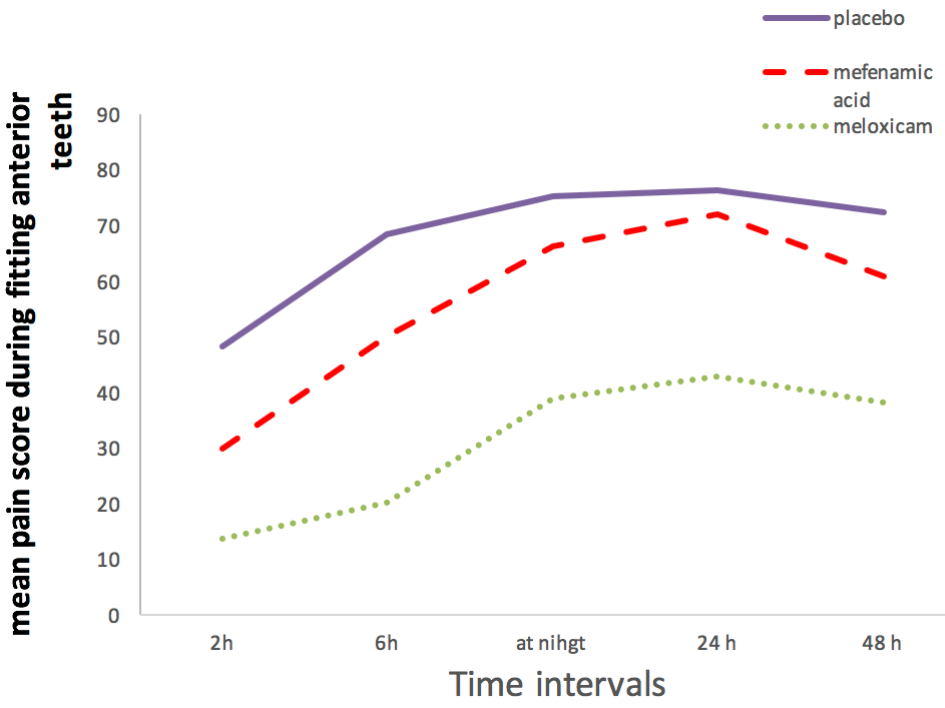

Fig. 3: Comparison of the mean pain scores on VAS among the three study groups over time on fitting the anterior teeth

Differences in Pain score between experimental groups in "Pain on Fitting the back teeth":

On measuring the differences in pain experienced on fitting the back teeth; the placebo group showed highly significant ($P < 0.001$) higher pain scores than the mefenamic acid and the meloxicam groups at all different time intervals, while the meloxicam group was with the least pain scores.

This study showed that the mean pain scores in fitting on the back teeth were less than other experimental group (Figure 4; Table 2).

Pain level in all three experimental groups increased gradually to reaches its peak level at 24 hours after separators placement, and then decreased gradually from the peak at 48 hours after separators placement.

At all-time intervals patients in the placebo group showed the highest mean pain scores, while patients on meloxicam showed the lowest mean pain scores (Table 2).

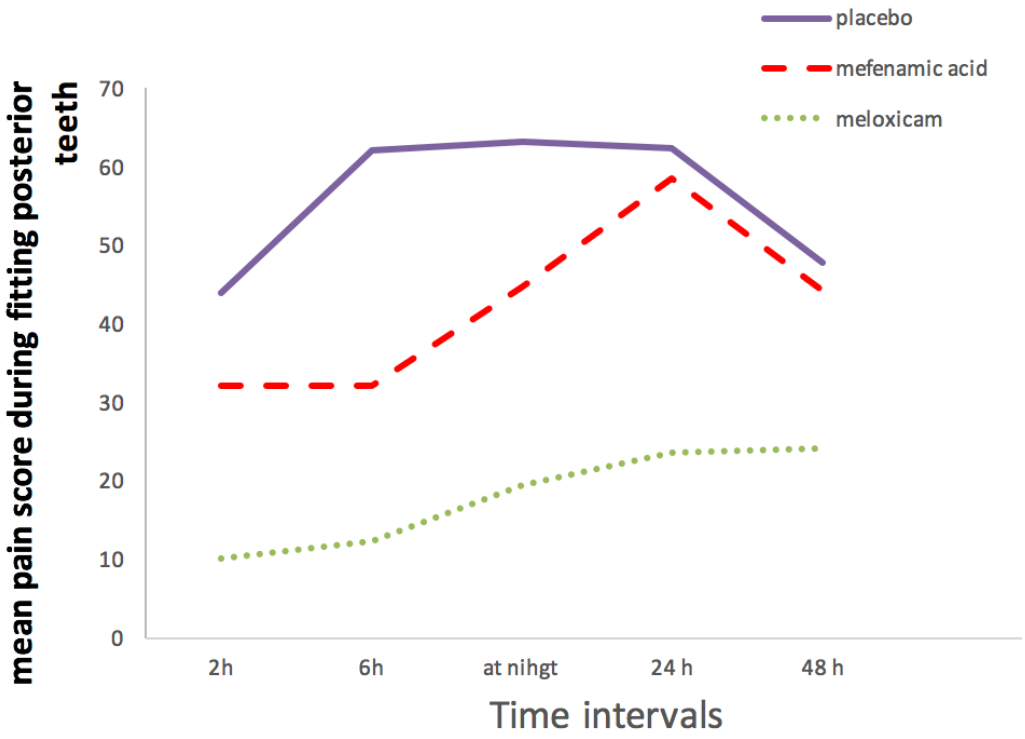

Fig. 4: Comparison of the mean pain scores on VAS among the three study groups over time on fitting the posterior teeth

DISCUSSION

Pain relief in dentistry has been fairly well studied in the literatures but the management of pain in patients receive orthodontic treatment is less well known.

As clinicians we are always asked whether it will be benefit for the patients to take analgesics during orthodontic treatment or not. Some studies have shown that pretreatment doses of NSAIDs may help to reduce the amount of pain experienced immediately after treatment [18-20].

This study was done with the aim to compare the efficacy of two analgesics, mefenamic acid and meloxicam in their standard doses in the management of orthodontic pain.as a preemptive analgesic one hour before the separator placement in the orthodontic patient.

In this study, the effect of preemptive administration of placebo, mefanamic acid, and meloxicam in controlling post-separator pain was evaluated and compared using visual analog scale (VAS). VAS is generally accepted to be the most reliable and accurate valid tool (instrument) for measuring acute and chronic pain, and is more sensitive for measuring positive responses to treatment compared to verbal descriptors [6,17,21].

Since the data and information in this study were collected by questionnaires, the questionnaire was translated from simple Arabic words to choice of the most suitable score in the visual analog scale (VAS).

The nature of pain caused by orthodontic treatment is not completely known. Dustman suggested that such a pain is caused by a combination of pressure, ischemia, inflammation and edema [5].

The results of this study show that pain levels increased from 2 hours to the maximum in the first 24 hours and then gradually declined from peak pain scores at 48 hours after separator placement. This finding is in accordance with those of previous investigations [7, 8, 22, 24, 25, 26].

Similar to other studies evaluating orthodontic pain level, the greatest reported pain occurred on biting and chewing rather than at fitting posterior teeth [22, 25, 25, 26, 27].

This is because orthodontic pain occurs as a result of compression, inflammation, and edema in the periodontal ligament, and there is greater compression during function in the periodontal ligament (PDL) [7, 27].

The results of this study revealed that patients in the meloxicam group reported the lowest pain scores in various conditions and different times, which is significant until 48 hours after separator placement compared to the other groups. This finding may be due to the absorption, selectivity and the high bioavailability of the drug. Meloxicam has a long mean half-life and, hence, has a longer duration of action than mefenamic acid thus provided pain relief for a longer duration, until the second day. [20]

There are two major concerns with using NSAIDs to manage orthodontic pain by inhibiting COX activity and thus prostaglandin production; one is that it may interfere with tooth movement, a number of animal studies [82,29] have demonstrated decreased rates of tooth movement with NSAID administration. However, the use of NSAIDs is only of concern in chronic users and not when taken at modest doses over the 3–4 days following treatment.[11] The other is their gastrointestinal side effects, meloxicam is a relatively COX inhibitor with more inhibitory effects on COX_2 than COX_1. Via this inhibitory effect on COX_2, it could be effective in orthodontic pain control. In addition, COX_1 inhibition is responsible for the adverse gastric effects of non-selective NSAIDs [30]. It has been shown that meloxicam doses ≤15 mg decreased the incidence of gastrointestinal side effects such as perforation, ulceration, and bleeding than non-selective NSAIDs [31].

There has been an increased concern regarding the risk of cardiovascular thrombotic event associated with the administration of selective NSAIDs [13, 24]. However, it seems that meloxicam is relatively safer compared to other medications of this class of NSAIDs; specifically in lower doses such as what was used in the current study (a single 7.5 mg), the risk of cardiovascular events may be very low [15,32], So current study allowed us to state that meloxiam appears to be an analgesic of choice for orthodontic pain.

CONCLUSIONS

- Preoperative administration of 7.5 mg meloxicam one hour before separator placement decreases pain significantly at 2 hours; 6 hours; nighttime; 24 hours; and 2 days after separator placement in comparison to mefenamic acid or placebo.
- The use of 7.5 mg meloxicam as a single dose 1 hour preoperatively only is highly recommended for pain control after separator placement.
- The pain begins at the moment of separator placement and reaches its peak in 24 hours and then, gradually decreases until the 48 hours.
- The most severe pain was reported by patients during biting and the least on fitting on posterior teeth.

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A Review of Wound Closure Technique-Patient Morbidity Relationship After Wisdom Tooth Surgery

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ABSTRACT

Background: Surgical removal of impacted mandibular third molars is associated with various postoperative complications like pain, swelling and trismus. These complications influence the patients' quality of life in the week following surgery. Many surgical interventions had been attempted to limit these complications, among them is the wound closure techniques. This study carry out a literature review to evaluate the impact of these techniques on patient morbidity following LM3 surgery.

Materials and method: The MEDLINE and PubMed database was searched for the related studies. Twenty-one randomized prospective clinical trials, that measure part or all of the immediate outcomes, were included.

Results**:** Twenty-one randomized prospective clinical trials were evaluated. They described the partial wound closure modalities, including single suture technique, the excision of mucosa immediately distal to the second molar, the placement of drain, and a "sutureless" technique.

Conclusion: The role of wound closure techniques on immediate postoperative sequlae revealed a varied opinion among researchers.

KEYWORDS:

Wisdom tooth surgery, wound closure, pain, swelling, and trismus.

المستخلص

الهدف من الدراسة: القلع الجراحي للأرحاء السفلية الثالثة المطمورة يرتبط بمضاعفات ما بعد الجراحة مثل الألم والتورم وضزز. هذه المضاعفات تؤثر نوعية حياة المرضى في الأسبوع التالي للعملية جراحية. وقد حاولت العديد من المداخلات الجراحية للحد من هذه المضاعفات، من بينها تقانة إغلاق الجرح. هذه الدراسة تشمل مراجعة للبحوث ذات الصلة لتقييم تأثير هذه التقانات على إعتلال المرضى بعد الجراحة المذكورة اعلاه.

المواد والأسلوب: تم تفتيش قاعدة بيانات MEDLINE و PubMed للدراسات ذات الصلة، أدرجت واحد وعشرين تجربة سريرية عشوائية والتي تعنى ببعض او كل مضاعفات ما بعد الجراحة.

النتائج: تم تقييم واحد وعشرون من التجارب السريرية العشوائية والتي درست عدة طرائق إغلاق جزئية للجروح، بما في ذلك تقانة الخياطة الواحدة، واستئصال الغشاء المخاطي بعد الأرحاء الثانية، ووضع هجرة، وتقنية «انعدام الغرز».

الخلاصة: دور تقانات اغلاق الجروح على المضاعفات المباشرة ما بعد الجراحة كشفت عن وجود رأي متنوع بين الباحثين.

INTRODUCTION

The removal of impacted mandibular third molars (LM3) is the most performed procedure in oral and maxillofacial surgical practices [(1)]. It involves trauma to the soft and hard tissues, which results in various postoperative complications like pain, swelling and trismus. These complications considered as immediate postoperative outcomes that influence the patients' quality of life in the week following surgery [(2)]. Therefore, reducing these complications becomes imperative.

The severity of pain usually peaks within several hours after surgery and may last for several days or more. Facial swelling may also alarm patients and typically peaks at around one or two days before subsiding over the subsequent days. Trismus results from inflammation of the muscles that move the jaw and may persist for more than a few days causing concern and difficulty in eating for about two weeks or more [(3)].

Many surgical interventions had been attempted to limit these complications [(4, 5, 6, 7, 8, 9, and10)]. Among them is the wound closure technique that present with different modalities regarding primary and secondary closure techniques. Primary closure of third molar flaps, the socket is covered and sealed hermetically by a mucosa flap. In the secondary closure technique, the socket remains in communication with the oral cavity to facilitate drainage of inflammatory products. Conflicting opinions have been expressed in published researches concerning these two types of healing [(11)].

This study carry out a literature review to evaluate the impact of these modalities on patient morbidity following LM3 surgery.

MATERIALS AND METHOD

The MEDLINE and PubMed database was searched for the related studies using the following keywords: wisdom tooth surgery, wound closure, patient morbidity, pain, swelling, and trismus. Twenty-one randomized prospective clinical trials, that measure part or all of the immediate outcomes, were included.

RESULTS

The partial closure technique is also described as, secondary closure, and secondary healing by different investigators. Several methods had been described to achieve partial closure, including single suture technique [(12, 13, 14, 15 and 16)], the excision of mucosa immediately distal to the second molar [(6, 8, 11, 17, 18, 19,

and 20), the placement of drain (7, 21, 22, 23, 24, and 25), and a "sutureless" technique in which no form of suturing is performed (26, 27, and 28).

Single suture technique

In the single suture technique, a single suture was placed at the distal relieving incision while in multiple suture technique; the sutures were placed at the interdental papilla between the second and third molars and at the distal relieving incision (Fig.1 and 2).

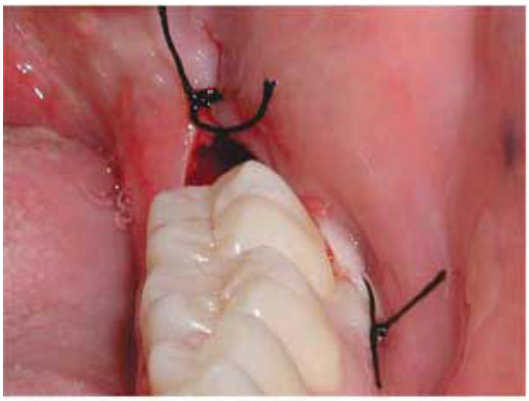

Fig.1: Wound closure by single suture technique: Healing by second intention (14).

Fig.2: Wound closure by multiple suture technique: Healing by first intention (14).

Five studies were included. Researchers (12, 13, and 14) founded a significant difference in term of pain, swelling and trismus between the two techniques favoring the single suture techniques. In another study, researchers revealed no statistical significant difference between the two techniques (15). Anighoro et al. (16) showed a significant reduction in postoperative pain and trismus in single suture technique, however, no difference in the values of facial swelling when compared with multiple suturing technique. The summery of the above studies seen in table (1).

Table 1. Influence of suturing technique on postoperative secondary outcomes.

Study	*Study design*	*Sample size*	*Age (Mean)*	*Technique*	*Outcomes*	*Results*
Osunde OD et al. (12)	Parallel group RCT	25	18-38 (26)	Single/ Multiple suture	Pain Swelling Trismus	Significant (Favor gp.I)
Refo'a Y et al. (13).	Parallel group RCT	16	>18	Single/ Multiple suture	Pain Swelling Trismus	Significant (Favor gp.I)
Sanchis-Bielsa JM, et al. (14)	Split-mouth RCT	25	18-31	Single/ Multiple suture	Pain Swelling Trismus	Significant (Favor gp.I)
Gay-Escoda C et al. (15).	Split-mouth RCT	40	18-45 (25.2)	Single/ Multiple suture	Pain Swelling Trismus	Not significant
Anighoro EO et al. (16)	Parallel group RCT	60	18-40 (26.8)	Single/ Multiple suture	Pain Trismus Swelling	Significant (Favor gp.I) Not significant

Distal mucosal excision

In this technique, partial wound closure was achieved by cutting a wedge of mucosa; width 5–6 mm, distal to the second molar, allowing secondary

wound healing (Fig.3).

Fig.3: Distal mucosal excision: Healing by second intention (6).

Seven studies were included. Researchers [6, 8, 11, 17, 18, 19, and 20] founded a significant reduction in postoperative pain, swelling when this technique was used as compared with complete closure technique. In addition, Bello et al, [8] revealed a significant reduction in term of postoperative swelling favoring distal mucosal excision technique, however; its effect in reducing postoperative pain and trismus were not significant. In contrast, two studies [17 and 19] showed that trismus was reduced significantly utilizing the distal mucosal excision technique. Table (2) summarizes the included studies that evaluate the effect of mucosal excision technique on postoperative secondary outcomes.

Table 2. Influence of distal mucosal excision on postoperative secondary outcomes.

Study	Study design	Sample size	Age (Mean)	Technique	Outcomes	Results
Maria A et al.[17]	Parallel group RCT	30	18-40	Mucosal excision/ No mucosal excision	Pain Swelling Trismus	Significant (Favor gp.I)
Chaudhary M et al. [18]	Split-mouth RCT	12	20-30	Mucosal excision/ No mucosal excision	Pain Swelling	Significant (Favor gp.I)
Khande K et al.[19]	Parallel group RCT	30	25-30	Mucosal excision/ No mucosal excision	Pain Swelling Trismus	Significant (Favor gp.I)
Danda AK et al [11].	Split-mouth RCT	93	18-31 (24.3)	Mucosal excision/ No mucosal excision	Pain Swelling	Significant (Favor gp.I)
Kareem JJ [20]	Parallel group RCT	50	19-27	Mucosal excision/ No mucosal excision	Pain Swelling	Significant (Favor gp.I)
Pasqualini D et al [6]	Parallel group RCT	100	19-27	Mucosal excision/ No mucosal excision	Pain Swelling	Significant (Favor gp.I)
Bello SA et al [8]	Parallel group RCT	40/42	21-32 (26.8)	Mucosal excision/ No mucosal excision	Pain Swelling Trismus	Not significant Significant (Favor gp.I) Not significant

Placement of drains

In this technique, a tube drain or gauze drain partially submerged into the extraction socket to secure more drainage and to attain secondary wound healing.

Many studies founded no effect on drain placement in postoperative pain reduction [7, 21, 22 and 23], but one study [24] revealed positive drain effect in pain reduction when compared with wound healing without drain placement. When swelling complication was considered, the use of drain was seen to be beneficial through many studies [7, 21, 23, 24, and 25]. However, Akota et al, [22] recorded a non-significant effect of drain in reducing postoperative swelling. The effect of drain in the reduction of postoperative trismus was conflicting. Researchers documents its validity [7, 23 and 24] and others are not [21, 22 and 25]. The summery of the above studies seen in table (3).

Table 3. Influence of drain placement on postoperative secondary outcomes.

Study	Study design	Sample size	Age (Mean)	Technique	Outcomes	Results
Obimakinde OS et al. [24]...	Parallel group RCT	40	>18	Tube drain/ No drain	Pain Swelling Trismus	Significant (Favor gp.I)
Chukwuneke FN et al. [7].	Parallel group RCT	50	18-40 (26)	Tube drain/ No drain	Pain Swelling Trismus	Not significant Significant (Favor gp.I)
Cerqueira PR et al. [21]	Split-mouth RCT	35	>18	Tube drain/ No drain	Pain Swelling Trismus	Not significant Significant (Favor gp.I) Not significant
Sağlam AA [25].	Split-mouth RCT	13	18-39	Tube drain/ No drain	Swelling Trismus	Significant (Favor gp.I) Not significant
Akota I et al. [22]	Split-mouth RCT	26	>18	Gauze drain/ No drain	Pain Trismus Swelling	Not significant
Rakprasitkul S and Pairuchvej V [23].	Split-mouth RCT	23	>18	Tube drain/ No drain	Pain Swelling Trismus	Not significant Significant (Favor gp.I)

Sutureless technique

In the sutureless technique there is free flow of inflammatory exudates from the extraction sockets and allowing the wound to heal secondarily [26].

Three studies were included and the results showed that patients had significantly less postoperative pain, swelling, [26, 27and 28] and trismus[26] when no sutures were used. Table (4) summarizes the included studies that assess the impact of sutureless technique on postoperative secondary outcomes.

Table 4. Influence of sutureless technique on postoperative secondary outcomes.

Study	Study design	Sample size	Age (Mean)	Technique	Outcomes	Results
Hashemi HM et al. [26]	Split-mouth RCT	30	19-24 (22)	Sutureless/ Multiple suture	Pain Swelling	Significant (Favor gp.I)
Osunde OD et al. [27]	Parallel group RCT	40	18-38 (27.1)	Sutureless/ Multiple suture	Pain Swelling Trismus	Significant (Favor gp.I)
Kaskos HH [28]	Parallel group RCT	20	(25.05)	Sutureless/ Multiple suture	Pain Swelling	Significant (Favor gp.I)

DISCUSSION

Swelling, trismus and pain are the most important indicators following surgical extraction of impacted lower third molars [8, 9 and 11]. Wound closure technique is an operative factor that could influence the immediate postoperative pain, swelling, and trismus. It could therefore contribute to the patients' quality of life after surgical operations [8]. However, this observation is controversial issue between researchers. The reason for this discrepancy is unclear [16] and might be resulted from variations in the individual inflammatory response [12]. In addition, the ability of the surgeon [29] and the difficulty of the operation might influence the outcome of the surgery.

Wound closure technique does not appear to have an influence on the amount of chemical mediators or their stimulation of nerve endings or the interpretation in the central nervous system, which could explain the lack of positive influence of the wound closure technique on pain perception [8]. Nevertheless, the accumulation of inflammatory exudate and/ or hematoma increases pressure on nerve endings resulting in more pain perception.

Pain assessment is subjective and influenced by many factors such as the patient's age, sex, and previous experience of pain, pain threshold and tolerance, therefore, assessment of pain may be difficult [30].

Facial swelling could be due to accumulation of inflammatory exudate16 within facial tissues, hematoma collection [31]. or both. Partial wound closure, which ensures drainage, appears to minimize immediate postoperative edema, thereby contributing to a reduction in patient discomfort [32]. Facial swelling could be measured by different methods like flexible tape, photograph, and the visual analog scale.

Although they are simple, cost-effective and time-saving methods, but not as accurate as magnetic for the measurements of facial soft tissue volume [33].

Primary closure of the flap avoids suture dehiscence and improves wound healing [34 and 35]. In contrast, Pasqualini et al, [6] and Dubois et al, [32] wound dehiscence occurred within the first 1 week postoperatively, more frequently in sockets with total closure. Researchers founded a significantly higher incidence of reactionary bleeding was observed with partial closure when compared the total wound closure [8]. It has been postulated that total wound closure will be associated with a higher incidence of dry socket because of the non-self-cleansing nature of the socket. It acts like a 1-way valve that allows food debris to enter the socket but does not allow it to escape from the socket [32].

The possible reason for the lower pain, swelling, and trismus values recorded for the single suture technique might be differences in the retention of the inflammatory exudates which is less in the partial (single suture) closure, because more room is present for the release of the inflammatory exudates compared with the multiple suture technique [12].

Although mucosa excision found to improve patients' quality of life [17, 18, 19, and 20], it prolongs the duration of surgery and may cause more trauma to the patient [36] and this may have negative impact on periodontal healing in the distal surface of second molar.

Many studies showed the advantage of drain placement in reducing post-operative swelling [7, 21, 23, 24, and 25]. Chukwuneke et al, [7] recorded a greater pain score for patients who had undergone surgical closure with the insertion of drain, probably because of the irritating effect. In addition, the overall cost of surgery could be increased because of the additional cost of purchasing rubber drains. Insertion of a drain could prolong the duration of surgery and may present with more discomfort to the patient due to the presence of a foreign body in form of a tube or gauze inside the mouth for a varying period of 48 to 72 hours after surgery [36].

Waite and Cherala has described this technique [9]. They reported less pain because of free flow of inflammatory exudates from the extraction sockets [27]. The benefits of this technique are the lower cost, less operative time, less manipulation of soft tissue and hence, less postoperative morbidity. Moreover, it does not require additional hospital visits for removal of sutures [9 and 27]. A sutureless technique might, however, be limited to cases in which minimal incisions are used for third molar surgery [9 and 34]. The main drawback of suture-less is that healing may be delayed. In addition, there may be high potential for the formation of a periodontal pocket in relation to the adjacent second molar [27]. However, Hashemi et al, [26]) showed, after 6-month follow up, that secondary wound healing through sutureless technique did not increase the depth of the pocket around the second molar.

CONCLUSION

The role of wound closure technique on immediate postoperative sequlae revealed a varied opinion among researchers.

Single suture technique and the distal mucosal excision may be valuable in reducing some of postoperative complications.

A sutureless technique might be advantageous in cases in which minimal incisions are used.

However, a recent meta-analysis concludes that there are no significant differences on the outcome between complete and partial wound closure and it refers that the available studies are heterogeneous and do not produce high level of scientific evidence [37].

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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Prevalence and Correlations Between Temporomandibular Disorders in Dental Students Based on Diagnostic Criteria (Dc/Tmd), Parafunctions And Psychoemotional Stress

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ABSTRACT
The aim of the study was to assess the prevalence of temporomandibular disorders (TMD) and oral parafunctions, as well as their correlation with psychoemotional factors among dental students by using diagnostic criteria (DC/RDC).the sample's size of 407 students (169 males and 238 females) of dental college (100 fifth stage , 102 fourth stage ,99 third stage and 106 second stage) . Firstly students subjected for stress questionnaire (perceived stress scale -10) , secondly they subjected to different clinical and questionnaire measures according to diagnostic criteria of temporomandibular disorders DC/TMD (axis1) which have standerized series of diagnostic tests based on clinical signs and symptoms , finally the dental students subjected for oral parafunctions questionnaire using oral behavior checklist.the results obtained from this study showed that the prevalence of TMDs was significantly greater among students with oral parafunctions . We also observed that the prevalence of TMDs according to DC/TMD was higher among students with headache .in this study, the prevalence of psycho-emotional stress , TMD according to DC/TMD and oral parafunctions were higher in females than males and in fifth stage greater than other stages.the prevalence of TMDs according to DC/TMD were significantly higher among females than males.

KEYWORDS
Temporomandsibular Disorders, Diagnostic Criteria (DC/TMD), Oral parafunctions ,Psychoemotional stress

INTRODUCTION
The temporomandibular disorders (TMD) and oral parafunctions seem to be a frequent problem inmodern societie [1] . The etiopathology of the temporomandibular joints is related to muscles, teeth arches, and periodontium. Their main causes involve both pathophysiological and psychosocial factors [2]. In the literature, a significant impact of the psychoemotional factor is reported, comparable to the impact of other factors concerning physical health such as systemic diseases, malocclusions, loss of teeth, traumas, and microtraumas [3]. Stress, fatigue, anxiety, depression, sleep disorders, and a fast pace of life affect negatively the human psyche [4]. In those patients muscular related TMD is observed more often[5].Moreover different studies report that TMD coexists with other numerous disorders such as SAPHO syndrome (synovitis, acne, pustulosis, hyperostosis, and osteitis syndrome), fibromyalgia, back- or spine ache, chronic fatigue syndrome, spastic colons, sleep disorders, congenital defects, headaches, and arthritis . Many studies report that the symptoms of the masticatory system disorders are more frequent in women than in men [6]. This may result from biological differences, including hormonal ones, and also psychosocial factors . Stallman reports that student population lives more under stress than the general population and develops considerably often TMD and oral parafunctions . The aim of this epidemiological study is to assess the prevalence of temporomandibular disorders and oral parafunctions among dental students and their correlation with psychoemotional factors by using diagnostic criteria (DC/RDC).

MATERIAL AND METHODS
The sample's size of 407(169 male and 238 females) students of 100 fifth stage , 102 fourth stage, 99 third stage and 106 second stage of college of dentistry university of Mustansiriyah in Baghdad city were included this study over the period from December 2014 to April 2015 . The analysis was conducted by the diagnostic criteria for temporomandibular disorders (DC/TMD).This enables the standardization of the procedures of epidemiological studies, the unification of TMD diagnostic and exploratory criteria. The results of the study were based on the DC/TMD Axis I diagnostic criteria. Mental state of subjects was not assessed according to DC/TMD Axis II diagnoses. The students subjected to perceived stress scale -10 that identifies students with potential psycho- emotional problems. The perceived stress scale -10 which is self- administrated questionnaire. Then students were subjected to a combination of questionnaire measures and clinical examination to differentiate recommended evidence-based new DC/TMD protocol is appropriate for use in both clinical and research settings. More comprehensive instruments augment short and simple screening instruments for Axis I. These validated instruments allow for identification of patients with a range of simple to complex TMD presentations. A dual-axis Diagnostic Criteria for TMD (DC/TMD)

will provide evidence-based criteria for the clinician to use when assessing patients, and will facilitate communication, regarding consultations, referrals, and prognosis. Finally ,The students were subjected again to oral behavior checklist to determine oral parafunctions and oral habit that may have effect on the etiology of temporomandibular disorders .

RESULTS

This study revealed that most of students in the college of dentistry were under stress and the differences were significant (p-value=0.044) between male (41.5%) and female (58.5%). Questionnaires are usually used together information about the prevalence of TMD in population. When students filled the specific questionnaire of DC/TMD , they revealed that students with pain have significant differences with p-value 0.023 and higher percentage was 44% in fifth class then 36% in second class, 33% in third class and 24% in fourth class. Headache recorded higher percentage than those with pain include 50% in fifth class then 27.4% in fourth class ,31.3% in third class and 22.6% in second class and also had a highly significant differences with p-value 0.000, while the history of pain and headache was reported higher in 17.22% females than 15.3% males. In this study, there is significant differences between clicking and genders with p-value 0.003 and females with clicking (17.2%) recorded higher percentage than males (13.6%) with clicking ,it may result from hormonal or biological factors in addition to , stress more in females than males and it is contributing factor to TMD ., there is also significant difference between clicking and educational stages with p-value 0.008 and higher percentage among fifth stage (29%) due to psychological and emotional factors then (5.8%) in fourth stage ,(16.1%) in third stage and (12.2%) in second stage. Statistical difference between oral parafunctions and educational stages was highly significant with p-value 0.003 and higher percentage among fifth stage was (10%) then (4.9%) in fourth stage ,(3.03%) in third stage and (1.88%) in second stage.

Table 1 : Statistical Difference Between TMD and Genders

		pain disorders						*p-value*	*Sig.*
		none	*myalgia*	*myofascial pain with referral*	*right arthralgia*	*left arthralgia*	*headache attributed to tmd*		
gender	male	106	23	5	7	2	26	0.003	S
	female	112	41	28	15	9	33		

Table : Differences Between TMJ Disorders and oral parafunctions

		diagnoses Tmj disorders					*p-value*	*Sig.*
		none disc displacement (select one)	*with reduction*	*with reduction, with intermittent locking*	*without reduction, with limited opening*	*without reduction, without limited opening*		
grind teeth together during waking hours	YES	36	10	8	3	0	0.000	S
	NO	319	19	6	5	1		
bruxism	YES	145	20	9	8	1	0.000	S
	NO	210	9	5	0	0		

DISCUSSION

Temporomandibular disorders have a multifactorial etiopathogenesis. Several authors underline the influence of local factors on their development, while others underline that of systemic factors [7]. The importance of psychological factors, such as increased psychoemotional activity and stress, is also emphasized in literature describing the etiology of TMD and oral parafunctions [8]. In the presented study we also observed a significant role of psychoemotional factors in the TMD development. Easily excitable and emotionally burdened persons suffered significantly more often from TMD and oral parafunctions. It must be mentioned that the DC/ TMD Axis I protocol includes valid diagnostic criteria for differentiating the patients in this study into two groups, with and without TMD. Although this protocol served the scope of the study, generally enables the diagnosis of a limited number and common TMD. Stress is also an important factor contributing to the TMD development. The examined student population is particularly susceptible to the influence of this factor [9]. This study revealed that most of students in the college of dentistry were under stress and the differences were significant (p-value=0.044) between male (41.5%) and female (58.5%). We found that the female more than male in being upset with p-value 0.042 and the stressors may include a large number of duties, the pressure of getting a good education, an uncertain future, low income, living far away from home, and functioning in an alien environment.

CONCLUSION

- In all stages females showed greater prevalence of psycho-emotional stress than males
- Among students of fifth stage the prevalence of psycho-emotional stress was higher than other stages
- Oral parafunctions (bruxism , cheek/lips biting , objects biting and nail biting) showed greater prevalence in fifth stages than others

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Physiological Changes of Salivary Parameters in Patients with End Stage Renal Failure

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ABSTRACT

Background: Many oral diseases were recorded in ESRD patients including as gingivitis, tooth mobility, xerostomia, ammonia-like smell, mucosal pallor and lesions, malocclusion and an increased risk of dental erosion. Salivary changes among ESRD patients might be attributed to renal failure, use of multiple medications, vomiting, depressive mood and low oral health hygiene.

Aim of study: to assess the salivary changes of patients with end stage renal disease.To determine whether there is changes in salivary compositions and biochemical parameters (urea, creatinine, salivary PH) of patients with end stage renal failure.To find whether there is change in salivary trace element (zinc, copper).To identify whether there is change in salivary electrolyte (sodium, potassium, calcium, phosphate).Determine whether there is a change in salivary enzymes (amylase).

Subjects, Materials and Methods:A cross sectional study carried out in two dialysis centers in Baghdad (Al-Khadhimya Teaching Hospital and Al-Karamah Teaching Hospital) through a period from 1st of March to end of 30th of July, 2015 on convenient sample of 64 patients with end stage renal diseases and on 64 healthy controls. Salivary samples were collected by the researcher at the end of dialysis. Saliva was collected using the standard way of collection. The samples controlled to avoid drooling or swallowing. Whole saliva was collected under resting conditions in a quiet room. Duration was between 0900 and noon at least 1 h after dialysis. Each patient was asked to chew a piece of Arabic gum (0.5-0.7 gm) for one minute, all saliva was removed expectoration, chewing was continued for five minutes with the same piece of gum and saliva was collected in sterile screw capped bottle. Salivary urea and Amylase were analyzed by specific kits, while slivary PH was tested by a hand-held pH meter. Salivary trace elements of saliva were analyzed at the Poisoning Consultation Center/Specialized Surgeries hospital by flame atomic absorption following standardized procedure.

Results: Mean age of ESRD patients was 50.1±14.9 years, males were more than females. No significant difference was observed between patients and controls regarding age and gender.There was a highly significant difference in salivary Potassium, Sodium, Calcium, Copper, Urea and Amylase levels between ESRD patients and controls ($p<0.001$). There was a significant difference in salivary Zinc level between ESRD patients and controls ($p=0.02$). There was a significant association between increased age of ESRD patients and HT ($p=0.04$). A significant association was observed between HT among ESRD patients and high salivary Zinc level ($p=0.008$).

Conclusion: Abnormal high Salivary Urea concentration in ESRD patients are associated with or was high and it was a significant predictor of ESRD. This study showed that salivary minerals (Potassium, Sodium, Calcium and Copper) were more likely to be reduced in ESRD patients undergone hemodialysis.

KEYWORDS

Salivary Parameters, Renal Failure.

المستخلص

مقدمه: سجلت العديد من أمراض الفم في مرضى الداء الكلوي بمراحله الأخيرة بما في ذلك التهاب اللثة، وضعف الأسنان، وجفاف الفم، رائحة الامونيا، الآفات والشحوب المخاطية، سوء الإطباق وزيادة خطر تآكل الأسنان. قد يعزى التغييرات اللعابية لدى مرضى الداء الكلوي بمراحله الأخيرة إلى الفشل الكلوي، واستخدام الأدوية المتعددة، والتقيؤ، والمزاج الاكتئابي وانخفاض النظافة الصحية الفموية.

الهدف من الدراسة :لتقييم التغيرات اللعابية للمرضى الذين يعانون من المرض الكلوي في مراحله الاخيرة.

طرق ووسائل ومنهجية البحث :دراسة مقطعية في مركزين لغسيل الكلى في بغداد (مستشفى الكاظمية التعليمي ومستشفى الكرامة التعليمي) خلال الفترة من ١ مارس إلى نهاية ٣٠ يوليو ٢٠١٥ على عينة ملائمة من ٦٤ مريضا مصابا بالفشل الكلوي بمراحله الاخيرة وعلى ٦٤ من الاصحاء. تم جمع عينات من اللعاب من قبل الباحث في نهاية غسيل الكلى. وقد تم جمع اللعاب باستخدام طريقة قياسية لجمع العينات الخاضعة للرقابة لتجنب سيلان اللعاب أو البلع. تم جمع اللعاب كله في ظل ظروف مريحة في غرفة هادئة. وكانت الفترة الزمنية بين ٩ صباحاالى الظهر و ١ ساعة على الأقل بعد غسيل الكلى. طلب من كل مريض مضغ قطعة من الصمغ العربي (٠،٥-٠،٧ جم) لمدة دقيقة واحدة، وإزالة كافة اللعاب. وقد تم تحليل اليوريا والأميليز اللعابية من قبل تحاليل مختبرية محددة، في حين تم اختبار فحص هيدروجينية اللعاب الحص الهيدروجيني اليدوي. وقد تم تحليل العناصر النزرة اللعابية في مركز التسمم التابع لمستشفى الجراحات التخصصيةبواسطة جهاز الامتصاص الذري.

النتائج: كان متوسط عمر مرضى الداء الكلوي بمراحله الأخيرة ١٤،٩ ± ٥٠،١ سنة، وكان الذكور أكثر من الإناث. لم يلاحظ أي فرق كبير بين المرضى والاصحاء فيما يخص العمر والجنس. كان هناك اختلاف كبير جدا في مستوى البوتاسيوم، الصوديوم، الكالسيوم، النحاس، اليوريا ومستويات الأميليزاللعابية بين مرضى الداء الكلوي بمراحله الأخيرة والاصحاء (P <٠،٠٠١). كان هناك اختلاف كبير في مستوى الزنك اللعابي بين مرضى الداء الكلوي بمراحله الأخيرة والاصحاء (P = ٠،٠٢). كان هناك ارتباط كبير بين زيادة عمر مرضى الداء الكلوي بمراحله الأخيرة وارتفاع ضغط الدم (P = ٠،٠٠٤). ولوحظ وجود ارتباط كبير بين ارتفاع ضغط الدم بين مرضى الداء الكلوي بمراحله الأخيرة وارتفاع مستوى الزنك اللعابي لهؤلاء المرضى (P = ٠،٠٠٨).

الاستنتاج: يرتبط ارتفاع تركيز اليوريا اللعابي مع مرضى الداء الكلوي بمراحله الأخيرة وكان مؤشرا كبيرا على الداء الكلوي بمراحله الأخيرة. وأظهرت هذه الدراسة أن المعادن اللعابية (البوتاسيوم، الصوديوم، الكالسيوم والنحاس) كانت أكثر عرضة للانخفاض في مرضى الداء الكلوي بمراحله الأخيرة.

INTRODUCTION

Renal failure is a process in which the nephrons lose their functional capacity and failure in filtering waste products caused by many causes. The renal failure is mostly reversible but chronic renal failure (CRF) had a progressive course deteriorated towards end stage renal disease (ESRD), although the cause of the initial nephropathy disappears [1].

The saliva is a unique biological fluid that described as the "mirror of the body". It is produced by salivary glands and composed mainly of water (98%) and 2% other compounds (minerals, electrolytes, etc.) [2]. Many oral diseases were recorded among ESRD

patients as gingival inflammation, tooth mobility, dry mouth, bad smell, mucosal changes and high liability for dental abrasion. Salivary changes among ESRD patients might be attributed to renal insufficiency, use of medications, vomiting, depressive mood and low health hygiene [3,4].

There is no wide researches on long term effect of dialysis on oral health and saliva, although, many literatures reported the harmful effect of renal transplant on oral health [5].

The main symptom of renal failure is an electrolyte disturbance and break in albumin-creatinine ratio that affect directly on saliva. Some literatures reported the significance of oxidative stress during dialysis in responsibility of salivary changes [6].

SUBJECT, MATERIALS AND METHODS

The study included sixty four patients with end stage renal diseases diagnosed by specialist physician in Internal Medicine and treated regularly with dialysis. The patients were selected from two dialysis centers in Baghdad (Al-Khadhimya Teaching Hospital and Al-Karamah Teaching Hospital) as a convenient sample.

The study was carried out for period from 1st of March to end of 30th of July, 2015.The inclusion criteria of the study were end stage renal diseases patients with dialysis duration more than one month. The exclusion criteria were mental disorder, hematological disease, diabetes mellitus and use of drugs interfering with salivary metabolism. The data was collected by direct interview and fulfilling a prepared questionnaire. The questionnaire included the following information:

1. Sociodemographic characteristics: Age and gender.
2. Clinical findings: Hemodialysis duration and hypertension.
3. Salivary parameters investigation results: Salivary Potassium, Sodium, Calcium, Copper, Zinc, PH, Urea and Amylase results.

Control subjects were recruited from Baghdad Medical City, private clinics and some friends from systemically healthy people but that required treatment due to dental problems. All controls had no systemic illnesses, including diabetes mellitus and renal or liver disease.Duration of dialysis was 4 hours per session, with 300 – 350 mL/minute blood flow rate or with a dialysate flow of 500 mL/minute. Patients with active infection or that were on medication influencing salivary flow rate, such as antidepressant drugs, were excluded.

Study design

A cross sectional study carried out on patients and controls to asse-ss the relationship between salivary parameters and ESRD patients as shown in figure1.

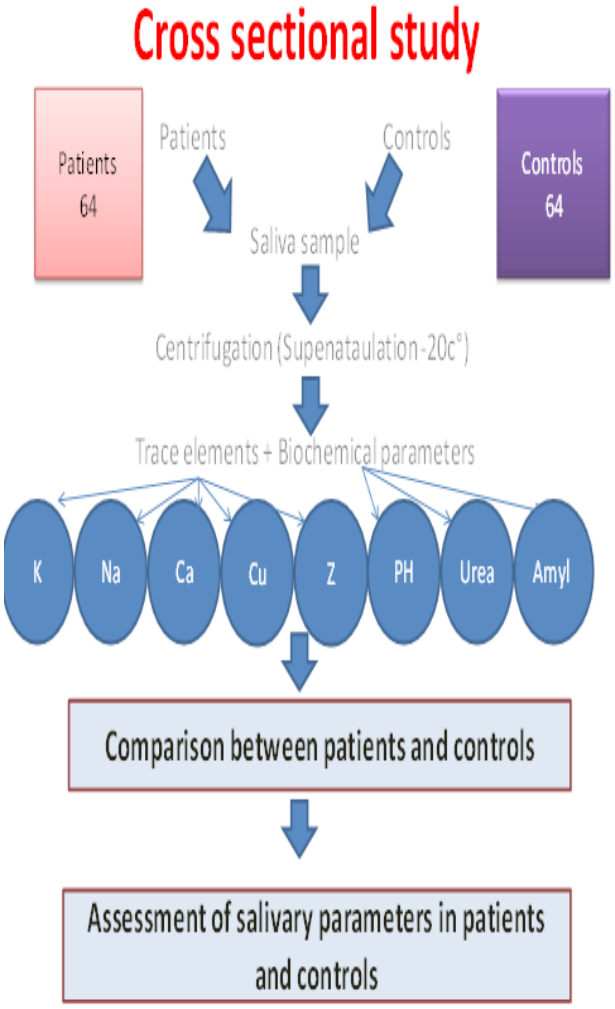

Figure 1: A schematic presentation of study design.

Materials used for salivary parameters estimation

1-Salivary Urea reagent

Table1:Randox kit.

Materials	*Concentration*
GAMMA GT (Liquid) (Mono Reagent)	0.05mmol/LL
CARBOXY (RX Daytona™)	0.05mmol/L
Distal water	1mmol/L

2-Salivary Amylase reagent

Table 2: Reagent of α-Amylase.

Materials	*Concentration*
MES PH 6	100mmol/L
2-chloro-4-nitrophe-nyl-α-D-maltotrisoside	2.25mmol/L
Sodium chloride	350mmol/L
Calcium acetate	6mmol/L
Potassium thiocyanate	900mmol/L
Sodium azide	0.9gm/L

3-Salivary PH

4-Salivary Potassium, Sodium, Zinc, Calcium and Copper

These parameters were tested through Atomic absorption Spectrophotometer use of special reagents of the device.

RESULTS

Biochemical parameters and trace elements

1. Salivary Potassium

Mean salivary potassium levels significantly ($p<0.001$) lowered in ESRD patients (0.2±0.03) than control group (0.3±0.02).

2. Salivary Sodium

The mean sodium level in saliva was highly significant lowered in 84.4% of patients with ESRD patients (0.4±0.06) as compared to control group (0.7±).

The results of the present study also showed that the mean salivary sodium level was significantly higher ($p< 0.05$) in male (0.52±0.02) than females (0.31±0.01) with ESRD.

3. Salivary Calcium

The results showed that the mean salivary Calcium level is low 67.2% of ESRD patients 1.7±0.2ml/dl as compared to zero in control group(3.076±٠,٤٣٠) with highly significant differences salivary calcium level between ESRD patients and control group (p<٠,٠١).

The current results revealed that the mean salivary calcium level was significantly higher ($p<0.05$) in males (1.9 ± 0.82) than females (1.5 ± 0.61) with ESRD.

4. Salivary Copper

The mean salivary Copper level is low in 92% of ESRD patients (1.8±0.4µgm/dl), when compared to4.8%control group. A highly significant difference was observed insalivary Copper level between ESRD patients and controls ($p<0.01$).

5. Salivary Zinc

The mean salivary Zinc level of ESRD patients was 4.5±1.3µgm/dl, 7.8% of them had low salivary Zinc level and mean salivary Zinc level of controls was 3.9±1.5µgm/dl, all of controls had normal salivary Zinc level. There was a statistically significant difference in salivary Zinc level between ESRD patients and controls ($p=0.049$).

6. Salivary PH

The mean PH of ESRD patients was 7.9±0.4, most of ESRD patients had basic salivary PH, 4 ESRD patients had neutral salivary PH and two patients had acidic PH, on other hand, mean salivary PH of controls was 7.8±0.3, the majority of controls had basic salivary PH and only 4 controls had neutral PH. No significant difference in salivary PH was observed between ESRD patients and controls ($p=0.285$).

7. Salivary Urea

The mean salivary Urea of ESRD patients was 0.6±0.2; range was 1.1, while mean of salivary Urea for controls was 0.2±0.06 with range as 0.2. There was a highly significant difference in salivary urea between ESRD patients and controls ($p<0.01$).

8. Salivary Amylase

The mean of salivary Amylase among ESRD patients was 61467.8±29943. For controls, mean salivary Amylase was 43721.8±14131.6. There was a highly significant difference in salivary Amylase between ESRD patients and controls (p=**0.030**).

DISCUSSION

Wide range of oral manifestations have been found among ESRD patients like, gingivitis, xerostomia, ammonia-like smell, mucosal pallor and lesions, tooth mobility, malocclusion and high risk of dental erosion caused by frequent regurgitation. Systemic and salivary imbalance caused by chronic renal failure, multiple medications, vomiting and low self-hygiene may affect oral health in these patients [8].

The Current results found that half of ESRD patients had dialysis duration more than one year. This finding came in agreement with finding of Al Wakeel*et al* (2002) [8] in Saudi Arabia who reported that mean duration of dialysis 5.7 years. The recovery of renal function in patients with prolonged hemodialysis is reported to be a relatively uncommon occurrence. Large observational cohorts of ESRD patients started on long-term hemodialysis from different parts of the world reported renal recovery rates of as low as 1%-2.4% [9].

In this study, Hypertension was present among 64.1% of ESRD patients. Also this finding is consistent with results of Silva *et al* (2012)[10] who observed that HT is the main cardiovascular risk factor associated with Brazilian ESRD.

Current study revealed that salivary Potassium level in ESRD patients was significantly lower than salivary Potassium level of control group. In contrast to study of Manley *et al* (2012)[11] who were found significant higher salivary potassium among Australian ESRD patients.

The current studyresults found highly significant difference ($p<0.001$) in salivary Calcium levels between ESRD patients and control, while Abdulla *et al*(2012)didn't find significant difference in the salivary Calcium between ESRD patients and control

groups.

Current results about lower salivary Calcium in ESRD patients are inconsistent with results of Manley *et al* (2012)[11] in Australia that didn't find significant difference in salivary Calcium between ESRD patients and healthy controls. The difference may be due to that ESRD patients in Austraian study were not on pre-hemodialysis phase and our patients were on post-hemodialysis phase.

Current results revealed that there were highly significant differences in the mean salivary Copper of ESRD patients. Our study was the first study exploring salivary Copper among ESRD patients in Iraq. This findings are close to results of Abdellatifet *al*(2011) [12]in Egyptwho observed deficient salivary Copper in ESRD children.

The results of the present study appeared that the salivary PH of ESRD patients and control were basic (mean=7.9, 7.8) respectively with no significant difference between the two groups regarding PH. This finding is consistent with the findings of results of(Bayraktar*et al*, 2009)[13] inTurkey who found that highly significant association between ESRD patients and increased salivary PH.Fortunately,the high salivary PH in ESRD patients decreases prevalence of dental caries among them [14].

According to mean of means salivary Urea there was a highly significant difference between ESRD patients and control group regarding salivary Urea ($p<0.001$). This is similar to findings of previous Iraq study (Abdulla*et al*, 2012)who showed that highly significant difference between patients that includes ESRD, hemodyalis and controls regarding salivary urea. Also Aliet*al*(2013) [15] in Indiareported highly significant increaseof salivary Urea among ESRD patients.

The findings of this study were clarified that salivary Amylase of ESRD patients significantly higher than mean salivary Amylase of control group. This is similar to results of (Tomás*et al*, 2008)[1] in Portugal patients with chronic renal failure (ESRD) compared to healthy controls when measured salivary biochemical parameters of both groups and found highly significant difference in salivary Amylase level between ESRD and control groups.

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