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Table of content

Changes in Pocket Depth, Clinical Attachment Level , and Alveolar Bone Height Distal to Lower Second Molar After Impacted Lower Third Molar Removal

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Abstract

The purpose of this retrospective clinical and radiographic study was to evaluate the periodontal condition of mandibular second molar after surgical removal of adjacent impacted mandibular third molars in patients who had undergone a unilateral, partially or fully impacted third molar extraction, at the Outpatient Clinic, Department of Oral and Maxillofacial Surgery, College of dentistry, Hawler Medical University, between the years 2010 and 2011.The sample size was 40 healthy patient with inclusion criteria aged between 18-32 years old.

The same operator removed the impacted third molars in all patients. Periodontal measurements including periodontal probing depth PPD, clinical attachment level CAL,& alveolar bone height ABH were examined at distal surface of second molars before & 6 months after surgical removal of impacted mandibular third molars .OPG was taken for each patients pre& post operatively. The data were analyzed using SPSS soft ware, version 12(SPSS,Chicago,IL).A paired t- test was used to fined a significant changes in the three recorded variables about 6 months post operatively. The results from this study showed that a significant improvements in all the periodontal parameters after removal of impacted mandibular third molars.

Introduction

Third molars, the last teeth to erupt into the human dental arch, have been shown to be the most frequently impacted teeth in all human ethnicities.[1] The main factors contributing to impaction are an inadequate dental arch space [2] and erratic eruption paths.[3,4] Impacted third molars, like other impacted teeth, can lead to a variety of problems, such as pericoronitis and/or orofacial infection, caries and/or periodontitis of the adjacent tooth, root resorption of the adjacent tooth, cystic or neoplastic changes, orthodontic or prosthetic problems, or even temporomandibular joint symptoms.[5,6]

Extraction of third molar teeth is the most common surgical procedure performed in the oral cavity. Numerous indications and contraindications for surgical extraction of third molars have been outlined[7,8], one of which is the prevention and/or improvement of periodontal defects in adjacent second molars[9-11] Surgical extraction of the third molar must attempt to conserve or even lead to the regeneration of the periodontal tissues on the distal surface of the adjacent second molar. However, the regeneration of such periodontal tissues seems difficult to achieve, because it represents a complex biologic process that is affected by local oral conditions, such as plaque accumulation, the inflammation of periodontal tissue, and the angulations of the third molar and its positional relationship with the adjacent second molar [12]

Several conflicting findings have beenpublished in previous literature regarding the effects of impacted third molar extraction on theperiodontal health of the adjacent second molar;some have suggested improvement of periodontal status distal to adjacent secondmolar [13,14] contrarily, other studies demonstrated loss of attachment and reduction of alveolarbone height [15,16] Periodontal defects after third molar surgery often can be anticipated before surgery based on the patient's age and preoperative periodontal health.

Although there is controversy regarding the removal of asymptomatic third molars, it is generally accepted that prophylactic removal of deeply impacted third molars is contraindicated in older patients with good periodontal health[17]. In general, periodontal defects after third molar surgery are most likely to occur in older patients (> 35 years), especially if there is existing bone lossalong the distal aspect of the second molar and if periodontal lesions, which are commonly associated with partially erupted third molars. For these patients, it is not advisable to perform the extractions unless pathologic indications necessitate such surgery [18] Because there is still a lack of consensus in the scientific literature addressing the effect of the extraction of lower third molars on adjacent second molars and on periodontal health, the aim of this study was to evaluate the periodontal conditions of mandibular second molars after surgical extraction of adjacent impacted Mandibular third molars.

Patients and Methods

retrospective clinical and radiographicstudy was designed to evaluate patients who had undergone a unilateral, partiallyor fully impacted third molar extraction, at the Outpatient Clinic, Department of Oral and Maxillofacial Surgery, College of dentistry, Hawler Medical University, between the years 2010 and 2011. In this study, convenience sampling method was used and the total of sample size obtained was 60 patients ,but only 40 patients attained

6 months follow up visits. All these patients had been given informed consents. Patients had been examined clinically and radiographically pre and post surgical removal of lower impacted third molar. The criteria for patient inclusion were age range between 18-32 years old; availability of agood-quality pre-operative panoramic radiograph; patients' good oral hygiene. Exclusion criteria included pregnancy , patients with no adjacent mandibular second molar; patient with chronic periodontitis, , pre-existing medical conditions that may impair wound healing including diabetes ,immunosuppression caused by chronic steroid use, status-post organ transplantation or chemotherapy for malignant conditions, previous radiotherapy to the maxilla or mandible, liver or renal failure (including dialysis patients), no permanent address or phone number, failure to agree to return for follow-up, mentally retarded individuals. Smokers were also excluded.

Clinical examination and recording of periodontal parameters was performed by one examiner (KH M) preoperatively and six months after impacted third molar removal. Periodontal pocket depth (PPD)&clinical attachment level (CAL) in mm was measured at the distal surface of adjacent lower second molar at the distobuccal and distolingual sites, their scores were then averaged using Williamsperiodontal probe. The probe tip was inserted into the gingival sulcus parallel to the long axis of the tooth until a slight resistance was met. All measurements wererecorded to the nearest millimeter. Clinical attachment level (CAL) was defined as the measurements in a millimeter from the cement-enamel junction to the base of the pocket. Alveolar bone height (ABH) from the cement-enamel junction of the distal aspect of the second molar to the crest of the bone was measured on orthopantomogram radiographs (OPGs), using gutta percha as a measuring guide. The OPGs was scanned & digitalized .Measurements of bone height on digitized OPGs was made using Auto Cad program.

All lower third molars were extracted by the same surgeon (SHA)with patients under local anesthesia, (lidocaine 2% with 1:80:000 adrenaline). The surgeon raised a full-thickness flap with a vertical releasing incision mesial to the second molar. Bone was removed on the buccal and distal aspects of the third molar using a surgical bur under copious normal saline irrigation. The tooth was appropriately split and removed. To close the wound, No. 3-0silk suture was used. After 7 days, the suture was removed. Data was analysed using SPSS software, version 12 (SPSS, Chicago, IL). A paired t-test was used to find if there was any significant change in the three recorded variables six months postsurgery. Differences were considered statistically significant at $P<.05$.

Results

The periodontal pocket depth (in mm)at baseline and six months following surgical removal of the impacted third molars is shown in Table 1. The pocket depth showed a high significant reduction at both distolingual and distobuccal sites ($p<0.01$). The amount of reduction was approximately 1.64 mm ((±0.17mm) for the former and 1.78 mm ((±0.19 mm) for the later site. The clinical attachment level (in mm) preoperatively and six months postsurgery is shown in Table 2. There was a significant gain in clinical attachment level ($P<0.05$) at both distobuccal and distolingual sites. The amount of attachment gain was 1.23 mm ((±0.31mm) for the former and 1.09 mm (0.25mm) for the later site. Alveolar bone height (in mm) at the base line and six months postsurgery is shown in Table 3. The improvement in distal bone height was1.35 mm (±0.27) and was statistically significant.

Table 1: Mean periodontal pocket depth distal to lower second molar before and six months after impacted third molar removal. *highly significant

Periodontal Pocket Depth (mm)					
	Before surgery Mean ±SD	6 months post-surgery Mean ±SD	Difference Mean ±SD	T-value	*P* -value
Distobuccal	4.907±0.37	3.265±0.28	1.642±0.17	2.964	<0.01*
Distolingual	5.123±0.29	3.339±022	1.784±0.19	2.771	<0.01

Table 2: Mean clinical attachment level distal to lower second molar before and six months after impacted third molar removal. *significant

Clinical attachment level (mm)					
	Before surgery Mean ±SD	6months post-surgery Mean ±SD	Difference Mean ±SD	T-value	*P*–value
Distobuccal	3.480±0.22	2.253±0.27	1.227±0.31	2.226	<0.05*
Distolingual	3.579±0.26	2.496±0.18	1.083±0.25	2.108	<0.05

Table 3: Mean alveolar bone height distal to lower second molar before and six months after impacted third molar removal. *significant

Alveolar Bone Height (mm)					
	Before surgery Mean ±SD	6months post-surgery Mean ±SD	Difference Mean ±SD	T-value	*P*–value
	4.620±0.33	3.265±0.27	1.355±0.23	2.301	<0.05*

Discussion

Surgical management of impacted third molars, whether for prophylactic or symptomatic reasons, is a common procedure provided by oral and maxillofacial surgeons. However, the removal of asymptomatic third molars is not without controversy and debate.[19] Critics of this practice believe that among the postoperative sequel of this procedure is periodontal pocket formation on the distal aspect of the adjacent second molar.[10, 13]

The results of this study show significant improvements in all the periodontal parameters evaluated (PPD, CAL, and ABH) from baseline to the final evaluation six months after the extraction. These improvements would partially stem from the better plaque control and oral hygiene performed by the subject after third molar removal. With partially impacted teeth, their removal provides access for adequate cleansing of the distal aspect of the second molar and the existing periodontal attachment can be maintained or improved.

It is plausible that third molar removal would effectively decrease the surface area of the biofilm gingival interface, thus altering subgingival anaerobic conditions that facilitate colonization of pathogens and an immune response to the bacteria, and potentially improve the periodontal condition.

The results of many studies support our findings. Blakey *et al* [11] concluded that removal of mandibular third molars significantly improved the periodontal status of the distal surfaces of the second molars and was also positive in terms of overall periodontal health. Along the same lines,Krausz *et al* 16 reported that extraction of an impacted lower third molar resulted in a significant gain of alveolar bone height on the distal aspect of the adjacent second molar on the test side, whereas a slight degree of bone loss was noted on the control side.In addition , Kim *et al* [20] concluded that third molar extraction in periodontitis patients showed an improvement in periodontal status in contrast the patients group having third molar, therefore earlier a removal of third molar may minimize radiographic bone loss of the adjacent second-molar.Montero and Mazaglia [21] also found that initial periodontal breakdown established on the distal surfaces of the second molars and in the periodontal health of the 4 posterior sextants can be significantly-improved 1 year after surgical removal of the ipsilateral lower third molar.

In contrast, other studies have shown that impacted lower third molar removal have a negative impact on the periodontal status distal to adjacent second molar.Gröndahl and Lekholm[22] demonstrated no significant changes in alveolarbone height distal to the second molar afterimpacted lower third molar extraction. In theirstudy, the duration was 12 months while in ourstudy the duration was only six months. Kugelberg [23] showed similar results where there were no gross changes of ABH following third molar extraction. However, in his study,they compared the ABH at 2 and 4 years after extraction. Osborne *et al* .[24] and Quee *et al* .[25]had also shown that there were no significant changes in PPD following third molar extraction regardless of the age and sex of thepatients. Besides that, Dodson [26] conducted a review paper of eight articles about this topic. The inclusion criteria for thisreview paper were prospective cohort studies

or randomized clinical trials with follow-up periodsof 6 months or more. They found that clinicalattachment level and periodontal pocket depthon the distal side of second molar 6 months post removal of impacted eights were clinically insignificant.

This great variation in the opinion of researchers concerning the effect of surgical removal of impacted lower third molars on the periodontal status of adjacent second molars may be attributed to many factors. The sample size, duration of follow up visits, age of the patients, difficulty of surgical removal of third molar as well as amount of bone removed and type of flap design and suturing technique, all may be of importance. The oral hygiene of the patient and his commitment in dental home care,may also play a role.

Regarding age, Kugelberg *et al* [12] postulated that the effects of age on decreasing cellular immunity to dental plaque might underlie the discrepancies found between younger and older patients with regard to their periodontal responses after third molar removal. The importance of age in periodontal healing was also confirmed in a study by Kaminishi *et al* ,[27] who stated that patients aged 40 years or older have an increased risk of periodontal problems after the removal of third molars.

Another possible explanation for the difference in the results between studies might be attributed to the differences associated with PPD measurements distal to the second molars with a neighboring impacted third molar [28].

One criticism of the study design may be that the six month duration of follow-up was inadequate. However, we believe that longer duration of followup visits may need cooperation of patients as the majority may not attend for longer multiple visits if they have no complain. We also believe that longer duration visits may mask any changed that had occurred due to the influence of many other variables on periodontal status.

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The effects of high dose of iodine on the anatomical and histological features of the thyroid glands in rabbits during the prenatal and postnatal periods

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ABSTRACT

Background and objectives: Iodine is an essential nutrient for optimal thyroid function, the major health effects of concern with excess iodine ingestion are thyroid disorders. The purpose of this study was to investigate the effects of the toxic dose of iodine on the anatomical and histological features of the thyroid glands in rabbits during prenatal and postnatal periods.

Methods: Thirty two adult female rabbits were selected. Only sixteen female rabbits were given oral dose of iodine (11 mg/kg of body weight dissolved in 1ml of distilled water) twice daily for a period of one and a half month, then rabbit offspring's thyroid glands were studied anatomically and histologically.

Results: The study group showed significantly higher rate of thyroid glands developmental disturbances ($p<0.05$), significant increase in thyroid glands weight in the postnatal period only ($p<0.05$), and in the thyroid gland volume in the prenatal period at 21th day of gestation and postnatal periods ($p<0.05$). Histological analysis showed significant decrease in heights and increase in widths of follicular cells ($p<0.05$) in the study group at postnatal periods. The thyroid tissues at 21 th day of gestation in the control and study embryos were nearly similar in the diameter of the follicles, but the number of the follicles in the study group was significantly less than the control group ($p<0.05$). The study group in postnatal periods showed significantly lesser number of follicles but with larger diameter and the colloid showed cracking. A significant decrease in the blood vessels density was also seen ($p<0.05$).

Conclusion: High dose of iodine causes goitrogenic effect which may induces the blockade of hormone biosynthesis.

Key words: Iodine, thyroid gland, rabbit.

INTRODUCTION:

Iodine is an essential component of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). Thyroid hormones regulate many important biochemical reactions, including protein synthesis and enzymatic activity, and are critical determinants of metabolic activity [1]. Exposure to high amounts of iodine occurs via food [2], drinking water [3], medication [4] and iodized salt or iodinated oil [5]. Any increase in iodine intake will cause some increase in the incidence of hyperthyroidism in a previously iodine-deficient population. Doses of 30–250 ml of tincture of iodine (about 16–130 mg of total iodine per kg of body weight) have been reported to be fatal to human [6]. Hypothyroidism from excessive iodine intake is much more common than hyperthyroidism. Hypothyroidism is attributed to the prolonged suppression of thyroid hormone production as the result of excess iodine level [7].

Since the thyroid gland plays an important role, at least in later stages of development and growth, the onset and pattern of its biochemical (functional) differentiation in embryo and fetus have attracted much attention, as well as the interrelationship between the glands of mother and fetus in placental mammals. In pregnancy more iodine is required, to ensure maternal thyroid hormone (T4) production, and can be maintained at almost double that of the non-pregnant state. The fetus is entirely dependent on T4 transferred from the mother during the first and second trimesters and on iodine transfer for fetal thyroid hormone synthesis during the last trimester [8, 9].

This study is designed to investigate the effect of iodine toxicity on the gross anatomical features and histological picture of rabbit's thyroid gland during prenatal and postnatal periods in the study group compared with that of the control group.

MATERIALS AND METHODS:

The present study were done in the period between November/2010 and May/2011, and approved by the local scientific and ethical committee of the College of Dentistry in Hawler Medical University.

The total number of the animals used in this study was thirty two adult female rabbits (Oryctolagus cuniculus), weighing about (2.5-3kg), and not less than 10 months of age. The animals were acclimatized for one week to the laboratory conditions prior to experimental manipulation, with free access to standard laboratory diet and water and maintained in constant temperature controlled rooms (22±3 ◦C) with controlled lighting (12 h light/12 h dark).Pure iodine was extracted from povidone iodine solution [10]. After that a pilot study was conducted to find the standard highest tolerable, non lethal oral dose of iodine.

All females when paired with a male overnight are examined for a vaginal plug in the following morning. The day on which a vaginal plug was observed was designated day 0 of gestation. Sixteen female rabbits were given a constant oral dose of iodine (11 mg/kg of body weight dissolved in 1ml of distilled water)

twice daily, for a period of one and a half month (one week before mating , along the period of gestation and till the 7th day of the postnatal life).

All the samples were received an intramuscular injection of 0.5mg/kg of phenobarbital, after that a mixture of 50 mg/kg bodyweight of ketamine hydrochloride and 10 mg/kg body weight of xylazine 2% were used for subcutaneous injection in the neck. The fetal rabbits, in both control and study groups, were subdivided into four subgroups (four animals each) which represented those collected at the 14th and 21th day of gestation, at their birthday, and at 7th postnatal day.

The samples of the prenatal group were harvested by sacrificing the pregnant rabbits. After caesarean section, fetuses were collected; thyroid glands were exposed and examined for the presence of developmental disturbances, extracted from its position and directly weighed. Regarding the volume of the thyroid gland measurements, the mediolateral, anteroposterior and craniocaudal diameters of each lobe was recorded to represent the width, depth (thickness) and length respectively then the volume of each lobe was calculated using the formula of the volume of an ovoid (width*depth*length* π /6), then the summation of the volume of both lobes was determined to represent the total thyroid volume and the isthmus was not included in the volume calculation [11].

Thyroid samples were fixed in Bouin's solution, processed, sectioned, and stained by hematoxylin and eosin stain. Measurements of height and width of follicular epithelium were taken from at least 10 different randomly selected thyroid follicles for each rabbit (H&E, x 1000). The height of the cells was measured from the outside to inside edge and measured at four predetermined positions (12:00, 3:00, 6:00, and 9:00) [12]. The number of follicles and the vascular density were calculated in five randomly selected microscopical fields at 200x magnification, but the maximum diameter of the follicle was measured in ten follicles selected randomly (HE, x1000) using a stage and ocular micrometer. The means of all measurements were determined for each animal, and these measurements were also evaluated by two persons blind to the study.

Data were analyzed using Med-Calc statistical software. The data were non-normally distributed. Accordingly, non-parametric tests were used. Mann-Whitney U test was utilized to assess statistical significance in the median value between two groups and the P value of ≤ 0.05 was considered to be statistically significant.

RESULTS:

A.Anatomical features: In the prenatal period at 14th day of gestation, the thyroid gland anatomically was very hard to locate even with dissecting microscope and it was quiet impossible to study the presence of developmental disturbances, the weight and volume of the thyroid gland at this age. In other samples the thyroid was a bi-lobed organ located in the ventrolateral aspect of the trachea in the infrahyoid region just below the laryngeal prominence, near the cricoid cartilage with a narrow isthmus connecting the two lobes, and loose connective tissue was seen to enclose the gland which helps to fix the gland lobes to the tracheal tube. The absence of the isthmus was a developmental anomaly observed in 13 animals, (four in the control group and nine in the study group). Almost in all the samples, the lobes were extending from the 1st to the 3rd tracheal rings and only four cases in the study group seen extended to the 4th tracheal ring (Figure -1). No other developmental anomalies in the thyroid anatomy were noted. Statistical analysis showed significantly higher rate of developmental disturbances regarding the absence of isthmus or extension of the thyroid gland to the 4th tracheal ring associated with study group compared with that of the control group ($p<0.05$).

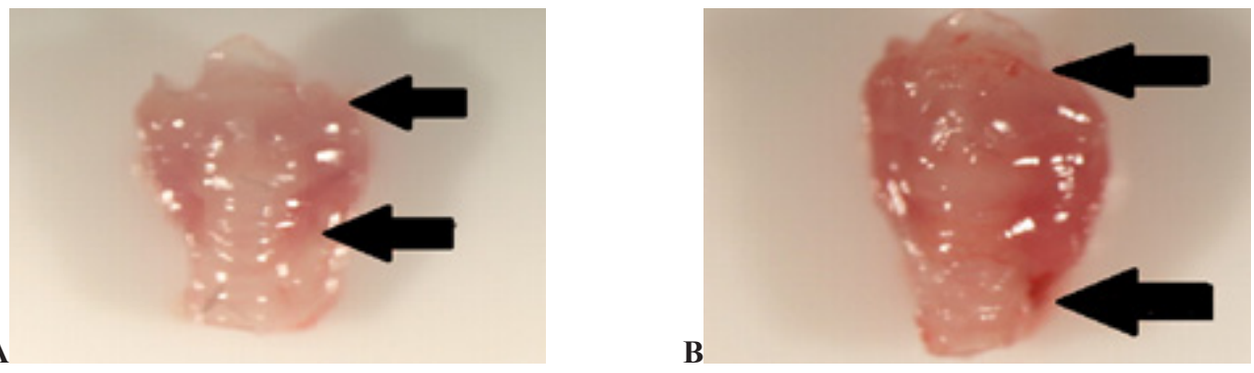


Figure- 1: The extension of the lobes of the thyroid gland. **A.** From the 1st to the 3rd tracheal ring (Arrows). **B.** To the 4th tracheal ring (Arrows).

The result also showed a significant increase in the thyroid gland weight in the postnatal period of the study group compared with that of the control group ($p<0.05$), but a non significant increase was found regarding the prenatal period at 21th day of gestation ($p>0.05$). No volume difference was observed between right and left lobes in all samples, but a significant increase in the thyroid gland volume in the prenatal period at 21th day of gestation and postnatal periods of the study group were seen compared with that of the control group ($p<0.05$) as seen in Table-1.

B. Histological features:

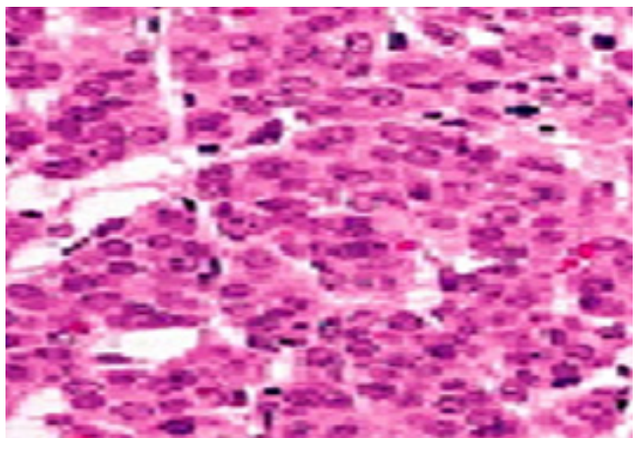
Follicular cell height and width: In the prenatal period at 14th day of gestation in both the control and study animals, the microscopical pictures showed that there were no significant microscopical signs of thyroid tissue development except for several clusters of columnar or cuboidal follicular cells that are not yet arranged to form follicles, yet although very little amount of first primordial follicle could be observed which indicates the starting point of thyroid tissue formation, development and differentiation (Figure-2).

In both the control and study groups, there were relatively no significant differences in the cells height and width in the prenatal groups, but there were significant decrease in the height and significant increase in the width of follicular cells ($p<0.05$) in the study group and the follicular cells appear flat compared with that of the control group which appear columnar or cuboidal shapes (Table-2). The toxic dose of iodine affected the thyroid gland of the study animals by inducing dysfunction and decreasing the cell height and increasing its width. The parafollicular cells population almost remained the same in both the control and study animal's thyroid samples.

Follicular number and diameter: Histological examination of the thyroid glands at the 21th day of gestation (Figure -3) showed that the glands in the control and study embryos were nearly similar in the diameter of the follicles, but the number of the follicles in the study group was significantly less than the control group ($p<0.05$). At birth, the thyroid tissues in the study group (Figure-4) significantly developed a lesser number of follicles but with a larger diameter ($p<0.05$). Seven days after birth, the thyroid tissue in the control group was normal in appearance and completely mature and the colloid was homogeneous and eosinophilic. In the study group, there were obvious differences in the affinity toward staining of the colloid with H&E stains in different follicles and even in the same follicle. Sometimes, different areas of the colloid in the same follicle showed obvious cracking. The number of follicles was significantly ($p<0.05$) less than that of the control group and the follicular diameter was significantly ($p<0.05$) larger with increase in the volume of colloid (Figure-5).

The analysis of H&E stained sections showed that the increased gland volume and weight in the postnatal samples of the study animals was associated with a widespread follicular enlargement and that the follicles were filled with colloid. This is accompanied by a decrease in the height of the follicular epithelial cells which appeared to be low cuboidal or even squamous in shape especially in the study samples that were aged seven days postnatal (Table-3).

The number of blood vessels: Statistical analysis showed a non significant increase in the thyroid tissue blood vessels number in the study group regarding the prenatal periods ($p>0.05$), but significant decrease was found in the postnatal periods compared with that of the control group ($p<0.05$), as seen in Table- 4.



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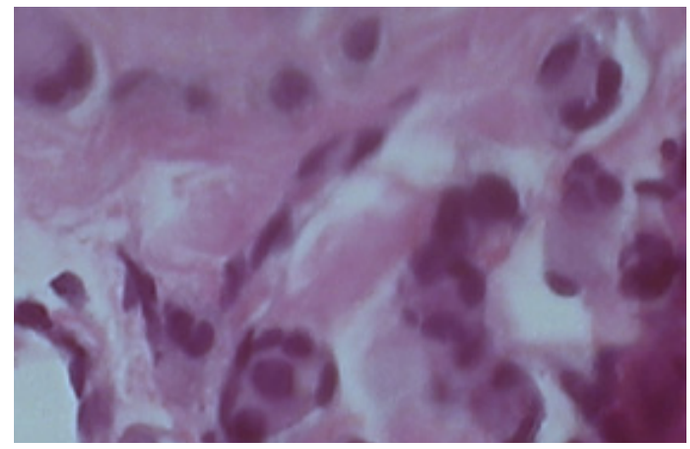
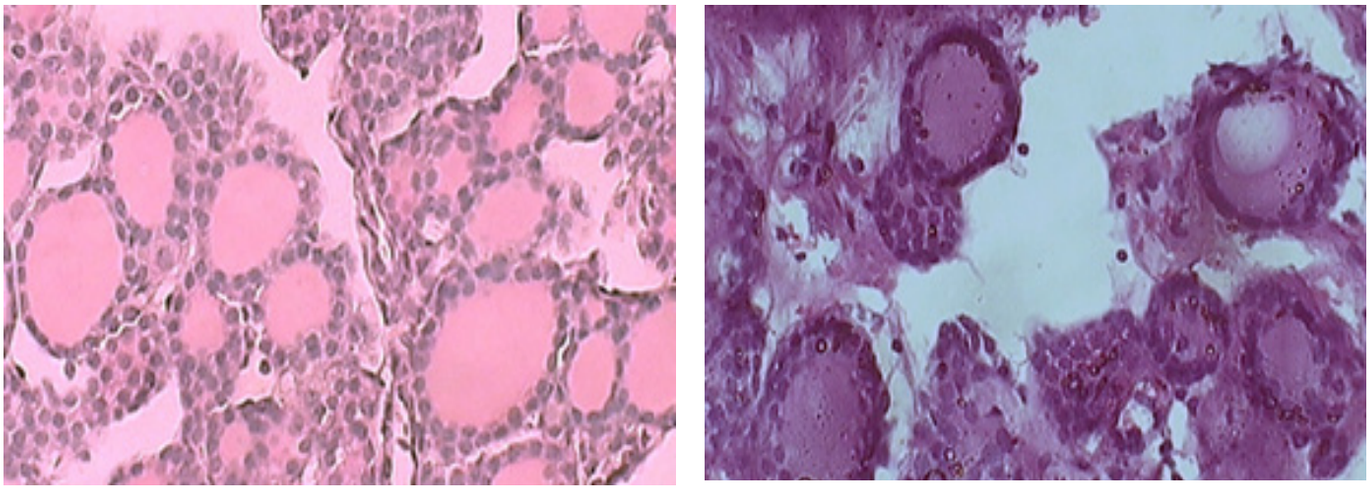


B

Figure -2: Thyroid tissue at 14th day of gestation **A.** Control group, the thyroid follicular cells are arranged in groups to form a primitive follicle (H&E, x 400). **B.** Study group, the follicular cells are less in number and irregularly distributed (H&E, x1000).



A B

Figure-3: Thyroid tissue at 21th day of gestation. **A.** Control group: The wall of the thyroid follicle build from cuboidal-shaped thyrocytes, the cavity of the thyroid follicle filled with colloid, and the parafollicular cells forming interfollicular clumps. **B.** Study group: Less number of thyroid follicles compared with that of the control group (H&E, x400).

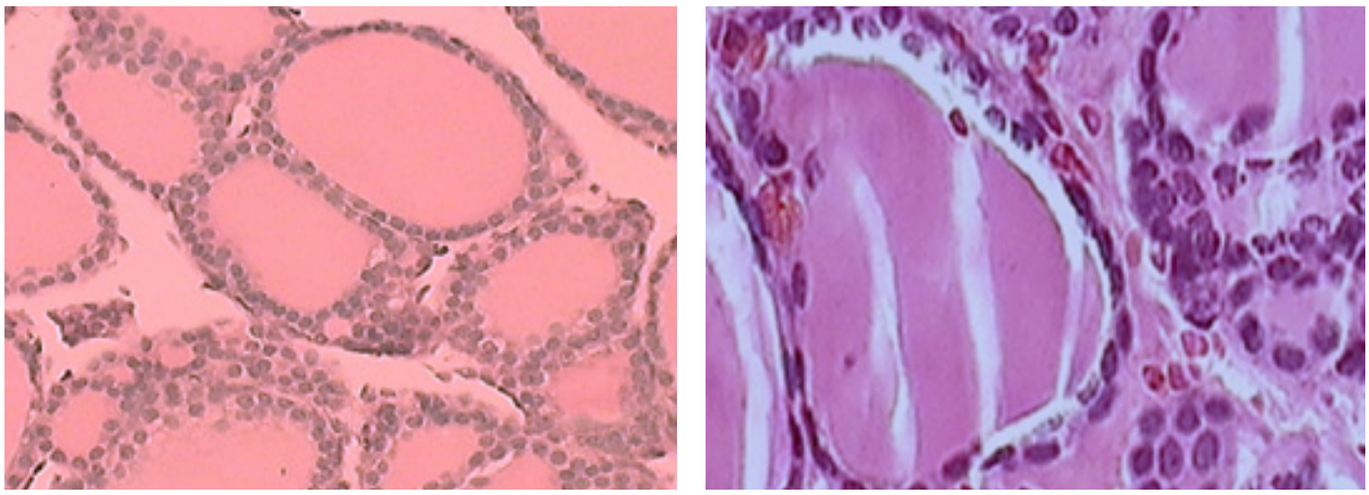


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Figure-4: Thyroid tissue at birth. **A.** Control group: The follicular cells are cuboidal and the colloid materials are homogenous. **B.** Study group: The follicular cells are flat shape and the colloid materials are not homogenous (H&E, x400).

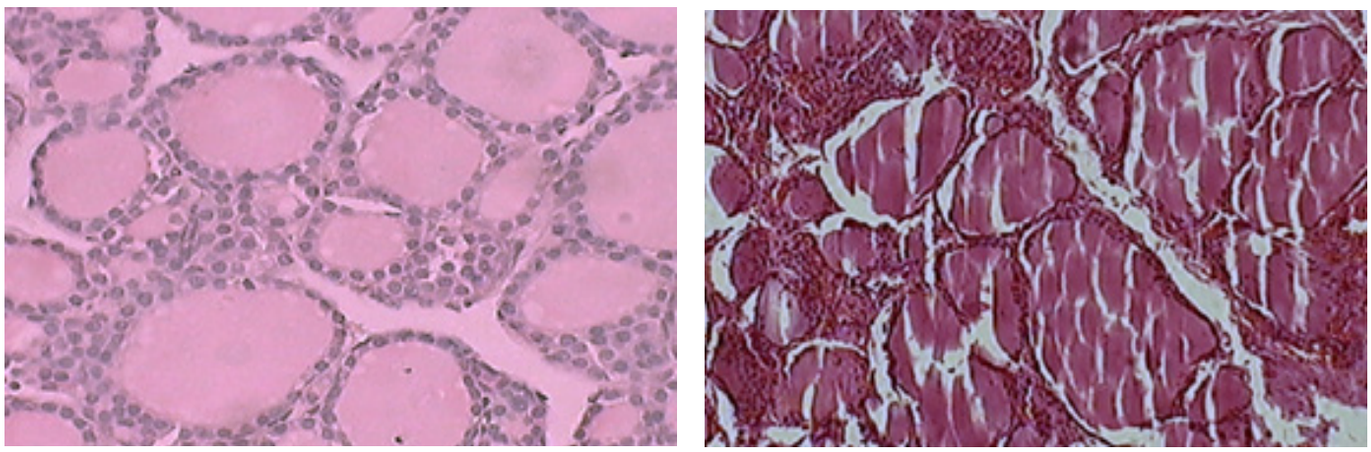

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Figure-5: Thyroid tissue of the control group seven days after birth. **A.** Control group: The follicular cells are cuboidal and the colloid materials are homogenous. **B.** Study group: The follicular cells are flat shape and the colloid materials are not homogenous with obvious cracking (H&E, x400)

Table -1: Thyroid glands weight (mg) and volume (mm3) in the control and study groups.

Age (Day)	Gender	Control group	Study group	P-value
21 days of gestation	Thyroid weight	61.925	64.124	0.248
	Thyroid volume	4.6	5.4	0.020
At birth	Thyroid weight	69.5	81.25	0.020
	Thyroid volume	5.80	9.0	0.020
7 days after birth	Thyroid weight	77.75	91.95	0.020
	Thyroid volume	6.65	12.90	0.020

Table-2: Follicular cell height (μm)and width (μm)of the thyroid tissues in the control and study groups.

Age (Day)		Control group	Study group	P-value
14 days of gestation	Average follicular cell height	1.25	1.24	0.248
	Average follicular cell width	1.475	1.52	0.052
21 days of gestation	Average follicular cell height	3.35	3.3	0.885
	Average follicular cell width	1.8	2.1	0.146
At birth	Average follicular cell height	3.85	2.5	0.020
	Average follicular cell width	2.05	3.6	0.020
7 days after birth	Average follicular cell height	5.5	2.32	0.020
	Average follicular cell width(μm)	2.45	3.4	0.020

Table -3: Median number and diameter of follicles in the thyroid gland in the control and study groups.

Age (Day)		Control group	Study group	*P*-value
21 days of gestation	Number of follicles	29.4	19.55	0.020
	Diameter of follicles	11.6	11.11	0.24
At birth	Number of follicles	44.75	32.8	0.020
	Diameter of follicles	14.55	27.1	0.020
7 days after birth	Number of follicles	52.45	43.82	0.020
	Diameter of follicles	18.96	32.3	0.020

Table-4: Median number of blood vessels in the thyroid gland of the control and study groups.

Age (Day)		Control group	Study group	P-value
14 days of gestation	No. of blood vessels	3.7	4.2	0.21
21 days of gestation	No. of blood vessels	6.2	6.5	0.23
At birth	No. of blood vessels	9.5	7.7	0.025
7 days after birth	No. of blood vessels	13.5	10.2	0.025

DISCUSSION:

Iodine toxicity significantly causes higher rate of developmental disturbances ($p<0.05$), this result comes in agreement with that of Nishiyama et al (2004) [13], and Xue *et al* (2006) [14]. The excessive iodine transferred by placenta may cause direct effect on the development of fetal thyroid.

In the study group, a non significant increase in weight of thyroid glands and significant increase in the volume were found regarding the prenatal period at 21th day of gestation ($p>0.05$), but this increases were significant in the postnatal period compared with that of the control group ($p<0.05$). This comes in agreement with the study of Guo et al (1991) and Zimmermann et al (2005) [15, 16]. In both the control and study groups, there were relatively non significant differences in the cells height and width in the prenatal groups, but a significant decrease in the height and significant increase in the width of follicular cells ($p<0.05$) in the study group were found, and the follicular cells appear flat and the follicles became distended with colloid, compared with that of the control group. Xue *et al* (2006) [14] studied the effect of toxic dose of iodine on the thyroid gland of the mouse and found the same result. The impairment of thyroid function caused by iodine toxicity leads to the inhibition of phagocytosis / pinocytosis of the colloid that contains thyroglobulin, causes it to accumulate in the follicular lumen and thereby diminishing the height of the follicular epithelium and increasing its width, and cause increasing the follicular diameter, and consequently the thyroid gland weight and volume were increased. This comes in agreement with the study of Zimmermann et al (2005) [16].

In the control and study embryos, the thyroid tissue at 21th day of gestation were nearly similar in the diameter of the follicles, but the number of the follicles in the study group was significantly less than the control group ($p<0.05$). At birth and seven days after birth the thyroid tissues of the study group showed a lesser number of follicles with larger diameter and the colloid showed obvious cracking. The decrease in the number of follicles and the increase in the size of follicles are due to increase in the colloid contents caused by a decrease in thyroid hormone secretion. This comes in agreement with results of Castillo *et al* (2003) [17].

Non significant increase in the thyroid tissue blood vessels number in the study group regarding the prenatal periods ($p>0.05$) was found, this might occur as a reaction of the body to the toxic dose of iodine, but significant decrease was found in the postnatal periods compared with that of the control group ($p<0.05$), and this might result due to the increased colloid contents of the thyroid follicles that raises intra thyroidal pressure and consequently reduces the vascularity in the area.

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A comparative study on the shaping ability of three endodontic rotary Nickel-Titanium systems and stainless- steel hand K-flexofile in simulated curved canals

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Dr. Hamid Kassim, B.D.S., M.Sc.

ABSTRACT

Background: The purpose of this study is to compare the Shaping ability of three rotary endodontic nickel-titanium systems (ProFile, CT and Protaper) with stainless steel hand K-flexofile in simulated curved canals at different levels, this include total canal diameter, outer and inner transportations and centering ratio (the ability of the instruments to remain centered in the shaped canals).

Materials and method: Eighty simulated curved canals made of clear polyester resin were used to assess instrumentation.The acrylic blocks were divided into four groups, 20 simulated canals for each group were enlarged from #10 to # 25. In the first three groups all NiTi rotary instruments were set into a permanent rotation with a 16:1 reduction handpiece powered by a torque-limited electric motor set at 300 rpm. All the instruments were used in a crown down manner using a gentle in-and-out (pecking) motion. In the fourth group the simulated canals were instrumented with stainless steel K-flexo-files by using balanced force technique. Each simulated canal was filled with a drawing ink using to increase the color contrast for photographic documentation. Photographs of the unprepared canals were taken by the aid of stereomicroscope and digital camera at magnification of 40 times. When instrumentation of the canals was completed, the canals were injected again with the drawing ink and the image procedure is repeated. Pre- and postoperative digital photographs of the resin blocks were accomplished using Adobe Photoshop CS2 software program. At this stage the amount of resin removed i.e. the difference between the canal configuration before and after instrumentation was determined for both the inner and the outer side of the curvature at five reference points.

Results: For total canal diameter there was highly significant difference among the four groups at all levels. For outer canal transportation there was highly significant difference among the four systems at all levels except at the second level where the differences were not significant. For inner transportation there was highly significant difference among the four groups at all levels. For centering ratio there was highly significant difference among the tested groups at all level.

Conclusion: K-flexofile scored the maximum canal diameter at the apical two levels. ProTaper prepared the largest canal diameter at all levels. In comparison with ProTaper, canals prepared by GT and ProFile maintained original curvature was better with less straightening. The ability of instruments to remain centered in prepared canals was significantly better in NiTi systems than K-flexofiles. ProTaper files have lower centering ability than GT and ProFile.

INTRODUCTION

The ideal preparation for the root canal is a funnel shaped form with the smallest diameter at the apex and the widest width at the orifice. This shaped form can be achieved either by hand or mechanical preparation. Various instrumentation techniques and endodontic instruments have been introduced in an attempt to reduce these problems aiming to provide the most favorable shaped preparation [1].The unique properties of nickel-titanium (NiTi) alloy, such as flexibility, have allowed the development of NiTi endodontic instruments in order to overcome the limitations imposed by stainless steel alloy [1]. The innovation of rotary NiTi instruments has totally changed the way of endodontics. Comparing these changes with K-files is truly dramatic. These changes are bringing the especially of endodontic practice into the twenty-first century with greater precision, fewer procedural errors, less discomfort to the patient, and faster case completions. Although there are many pitfalls on the road to consistent results, with proper use of the NiTi systems, endodontists will be able to improve the quality and esthetics of their endodontic obturations quickly [2].

The purpose of this study is to compare the shaping ability of three rotary endodontic nickel-titanium systems (ProFile, CT and Protaper) with stainless steel hand K-flexofile in simulated curved canals at different levels, this include:

a.Total canal diameter.

b.Outer and inner transportations.

c.Centering ratio (the ability of the instruments to remain centered in the shaped canals).

MATERIALS AND METHODS

Simulated curved canals made of clear polyester resin were used to assess instrumentation. The diameter and the taper of all simulated canals were equivalent to an ISO standard size 10 root canal instrument. The canals were 16 mm long, the straight part being 11 mm and the curved part 5 mm with angle of 40° [3]. (Fig.1).

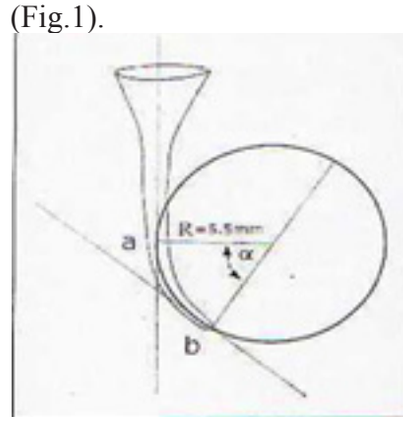

Fig.1: Angle and radius of canal curvature.

Preparation of artificial canals

Eighty acrylic blocks were divided into four groups, 20 simulated canals for each group were enlarged from #10 to # 25. The first penetration in the simulated Canal was performed with #10 K-file hand instrument to the full working length (16 mm). Patency of the resin blocks was checked with the same size after each sequence. Prior to use., each instrument was coated with glycerin to act as a lubricant and copious irrigation with tap Water was performed repeatedly before and after the use of each instrument using disposable syringes and 27 gauge tips.

Rotary NiTi instruments

In the first three groups all NiTi rotary instruments were set into a permanent rotation with a 16:1 reduction handpiece powered by a torque-limited electric motor set at 300 rpm and the torque at 1.2 Ncm. All the instruments were used in a crown down manner using a gentle in-and-out (pecking) motion until resistance was felt and changed for the next instrument.

Manual technique

In the fourth group the simulated canals were instrumented with stainless steel K-flexo-files by using balanced force technique described by Roan et al in 1985 [4] which continue until an apical stop of size 25 was achieved. Then stepping backs the preparation with ti 30, # 35 and # 40 files and used also in balanced force motion.

Assessment of canal preparation

Postoperative canal shape

Prior to their preparation, each simulated canal was filled with a drawing ink using a 27 gauge needle to increase the color contrast for photographic documentation. In order to achieve a standardized position of the resin blocks against the lens of the microscope, a holder was constructed from stone for this purpose with a hole. in the center in which the resin blocks could be placed and repositioned in exactly the same position. The central hole was covered with a transparent paper on which the five chosen levels were drawn and the artificial canal could be measured easily. Photographs of the unprepared canals were taken by the aid of stereomicroscope and digital camera at magnification of 40 times. One image on screen corresponded to 2 mm of the real canal length. Therefore eight images were needed to assemble the entire canal. Both X and Y coordinates on the microscope's nonius scale were recorded for each image, allowing repositioning and reproduction of the pictures at any given moment (i.e. pre- and postoperative). The images were standardized by securing the camera at a fixed distance from a microscope lens. After that the simulated, canals were cleaned using tap water with irrigating syringe. When instrumentation of the canals was completed, the canals were injected again with the drawing ink and the image procedure is repeated.

Pre- and postoperative digital photographs of the resin blocks were stored in a Pentium 4 computer and measurements were accomplished using Adobe Photoshop CS2 software program. At this stage the amount of resin removed. i.e. the difference between the canal configuration before and after instrumentation was determined for both the inner and the outer side of the curvature at five reference points, using a method described by Calberson et al in 2002 [5]. Al[measurements were made at right angles to the surface of the canal (Fig.-2).

- Point 1 (O): the canal orifice.
- Point 2 (HO): the point half-way from the beginning of the curve to the orifice.
- Point 3 (BC): the point where the canal deviates from the long axis of its coronal portion and is called the beginning of the curvature.
- Point 4 (AC): the point where the long axes of the coronal and the apical pen ions of the canal intersect and are called the apex of the curve.
- Point 5 (EP): the end point of preparation

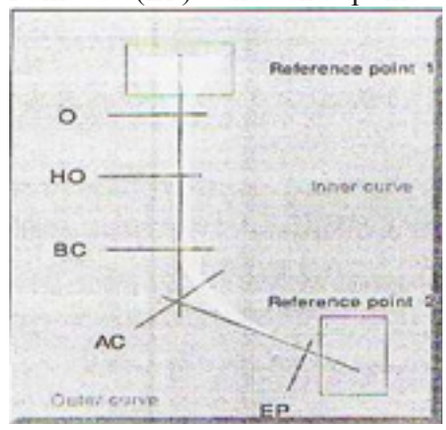

Fig.-2: The five levels of measurement

The mean centering ratio is a measure of the ability of the instrument to stay centered in the canal: the

smaller the ratio, the better the instrument remained centered in the canal. This ratio was calculated at each of the five points using the following formula [t6]:

X1-X2/Y

`X1: The maximum extent of canal movement in one direction.
X2: is the movement in the opposite direction.
Y: Is the diameter of the final canal preparation.

RESULTS

Postoperative canal shape

Total canal diameter

The mean values and the standard deviations of the total canal diameters after instrumentation at the five different levels examined for the four groups are presented in Table -1.

Table-1: Mean and standard deviation of post instrumentation total canals diameter (mm) for the four groups at the five levels.

System		O	HO	BC	AC	EP
PF	Mean	0.818	0.616	0.542	0.527	0.329
	SD±	0.012	0.006	0.048	0.034	0.027
GT	Mean	0.899	0.643	0.573	0.472	0.328
	GT	0.022	0.028	0.025	0.030	0.032
PT	Mean	0.974	0.969	0.784	0.631	0.351
		0.071	0.097	0.050	0.029	0.021
KO	Mean	0.829	0.777	0.731	0.750	0.368
	SD±	0.042	0.047	0.041	0.042	0.037
F-test		54.77	182.6	154.6	250.7	292.4
P-value		0.000 HS	0.000 HS	0.000 HS	0.000 HS	0.000 HS

ProTaper showed the largest canal diameter (0.974) at the first level and the smallest canal diameter (0.351) at the end point of preparation. GT showed the largest canal diameter (0.899) at the first level and the smallest canal diameter (0.328) at the end point of preparation. ProFile showed the largest canal diameter (0.818) at the first level and the smallest canal diameter (0.329) at the end point of preparation. K-flexofile showed the largest canal diameter (0.829) at the first level and the smallest canal diameter (0.368) at the end point of preparation. In general, the data confirm that; the NiTi files were flared the canals uniformly, being narrowest at their end point and widest at the orifice. The increase in width of the canals between each position varied from file to file.

Furthermore it became obvious that a proportion of canals which have been prepared by K-flexofile did not have a continuously tapering form often there were a relatively wider regions at the apex of the curve than the beginning of the curve, followed by a narrow regions towards the orifice.

The Student t-test (Table -2) revealed a highly significant difference between ProFile and GT at the first and fourth levels; while a significant difference was found at the second and third levels and not significant difference was found at the apex. By comparing ProFile and ProTaper, a highly significant difference was found at all levels except at the last one where the difference was significant. The difference between ProFile and K-flexofile was highly significant at all levels excluding the first one where the difference was not significant

Table -2 shown a significant difference between GT and ProTaper at the orifice and apex of the prepared canals and a highly significant difference at the other measuring points; While the difference between GT and K-flexofile was highly significant also there was a highly significant difference between ProTaper and K-flexofile along the canals, but not at the third level where the difference was significant.

Table -2: t-test of the post instrumentation total canals diameter for the four groups at the five levels

Groups	O		HO		BC		AC		EP	
	P-value	Sig	P-value	Sig	P-value	Sig	P-value	Sig	P-value	Sig
PF&GT	0.000	HS	0.005	S	0.017	S	0.000	HS	0.960	NS
PF&PT	0.000	HS	0.000	HS	0.000	HS	0.000	HS	0.008	S
PF&KO	0.280	NS	0.000	HS	0.000	HS	0.000	HS	0.000	HS
GT&PT	0.002	S	0.000	HS	0.000	HS	0.000	HS	0.015	S
GT&KO	0.000	HS	0.000	HS	0.000	HS	0.000	HS	0.000	HS
PT&KO	0.000	HS	0.000	HS	0.009	S	0.000	HS	0.000	HS

Outer transportation

The mean values and the standard deviations of outer canal transportation after instrumentation at the five measuring points are given in Table -3.

Table-3: Mean and standard deviation of outer transportation (mm) for the four groups at the five levels.

Groups	O		HO		BC		AC		EP	
	P-value	Sig	P-value	Sig	P-value	Sig	P-value	Sig	P-value	Sig
PF&GT	0.000	HS	0.005	S	0.430	NS	0.001	S	0.069	NS
PF&PT	0.000	HS	0.280	NS	0.019	S	0.000	HS	0.000	HS
PF&KO	0.032	S	0.910	NS	0.000	HS	0.000	HS	0.000	HS
GT&PT	0.000	HS	0.110	NS	0.003	S	0.000	HS	0.000	HS
GT&KO	0.000	HS	0.102	NS	0.000	HS	0.000	HS	0.000	HS
PT&KO	0.001	S	0.320	NS	0.001	S	0.000	HS	0.000	HS

GT showed the highest mean of outer transportation (0.260) at the first level and the smallest mean of outer transportation (0.119) at the end point of preparation while ProTaper showed the reverse, the highest mean of outer transportation (0.380) at the fourth level and the smallest mean of outer transportation (0.134) at the first level. ProFile showed the highest mean of outer transportation values (0.215) at the first and third levels and the smallest mean of outer transportation (0.128) at the end point of preparation. K¬flexofile showed the highest mean of outer transportation (0.484) at the fourth level and the smallest mean of outer transportation (0.127) at the third level.

By using the Student t-test (Table -4), a highly significant difference was found between ProFile and GT at the orifice and a significant difference at second and fourth levels; while no significant difference was found at the third and fifth levels. The relation between ProFile and ProTaper show a highly significant difference at the orifice, after curvature and end point; while it was not significant by the side of second measuring site and significant at the beginning of curvature. The variance between ProFile and K--flexofile was significant at the orifice and not significant next to the second level; while it was a highly significant at the other measuring points.

The Student t-test showed a highly significant difference between GT and ProTaper at the first, fourth and last part of preparation. At the second level there was no significant difference; while there was a significant difference at the third point of measurement. The difference between GT and K-flexofile was a highly significant at all measuring sites, but not at second level where the difference was not significant

Table-4: t-test of the outer transportation for the four groups at the five levels.

System		O	HO	BC	AC	EP
PF	Mean	0.215	0.206	0.215	0.205	0.128
	SD±	0.011	0.004	0.036	0.017	0.014
GT	Mean	0.260	0.218	0.222	0.188	0.119
	SD±	0.015	0.016	0.013	0.013	0.015
PT	Mean	0.134	0.184	0.178	0.380	0.200
	SD±	0.054	0.089	0.056	0.030	0.021
KO	Mean	0.198	0.205	0.127	0.484	0.465
	SD±	0.032	0.030	0.023	0.039	0.036
F-test		52.31	1.72	28.86	547.2	923.8
P-value		0.000 HS	0.000 HS	0.000 HS	0.000 HS	0.000 HS

Table -4 shown a significant difference between Pro-Taper and K-flexofile at the orifice and beginning of the curve; while there was no significant difference next to the second place; the relation was a highly significant difference after the curve and end point of instrumentation

Independent of the files used, all instruments removed material on the whole length of the outer side of canal. Canal shaped by the NiTi systems showed an almost regular removal of resin material on the outer side of the canals and remained more centered in the canals; while K-flexofile produced uneven pattern of resin removal along the outer aspect of the canals (Fig.3).

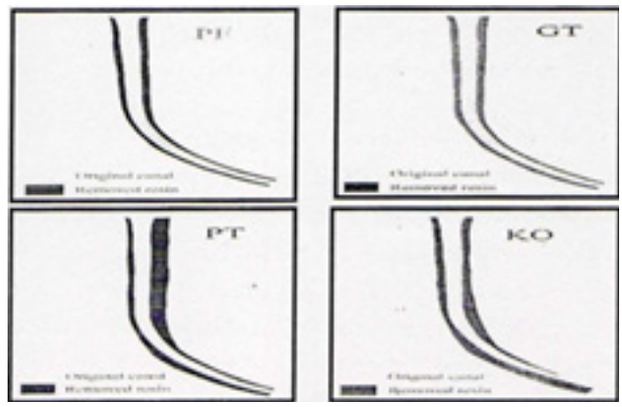

Fig.3: A plot of the mean changes in the canals as the results of preparation with different systems

Inner transportation

The mean values and the standard deviations of inner transportation after instrumentation at the five different levels examined for the four groups are listed in Table-5.

Table-5: Mean and standard deviation of inner transportation (mm) for the four groups at the five levels.

System		O	HO	BC	AC	EP
PF	Mean	0.202	0.239	0.213	0.195	0.100
	SD±	0.007	0.005	0.027	0.018	0.014
GT	Mean	0.240	0.255	0.229	0.174	0.106
	SD±	0.023	0.015	0.013	0.020	0.018
PT	Mean	0.441	0.613	0.486	0.141	0.050
	SD±	0.044	0.032	0.038	0.010	0.007
KO	Mean	0.230	0.396	0.478	0.152	0.000
	SD±	0.024	0.038	0.035	0.009	0.000
F-test		302.8	848.7	595.2	488.0	324.5
P-value		0.000 HS	0.000 HS	0.000 HS	0.000 HS	0.000 HS

At the second level, ProTaper showed the highest mean of inner transportation (0.613) and the smallest mean of inner transportation (0.050) at the end point of preparation but GT showed the highest mean of inner transportation (0.255) at the second level and the smallest mean of inner transportation (0.106) at the end point of preparation. Also ProFile showed the highest mean of inner transportation (0.239) at the second level and the smallest mean of inner transportation (0.100) at the end point of preparation while K-flexofile showed the highest mean of inner transportation (0.478) at the third level and did not touch the inner sides at the end point of preparation.

By using Student t-test (Table-6), a highly significant difference was found between ProFile and GT at the orifice and a significant difference at the second, third and fourth levels; while no significant difference was found at the last measuring point. The relation between ProFile and ProTaper was a highly significant difference at all levels. The variance between ProFile and K-flexofile was significant next to the orifice and a highly significant at the other measuring sites.

As well Student t-test showed a highly significant difference between GT and ProTaper at all levels. The difference between GT and K-flexofile was not significant at the first plane. The variation between these two instruments next to the second, third and last measuring points were highly significant; while there was significant difference at the forth level.

Also, Table-6 shown a highly significant difference between ProTaper and K-flexofile at the first, second and fifth tested points; while there was no significant difference next to the third level. After the curve the difference between these two groups was significant.

Table-6: t-test of the inner transportation for the four groups at different levels.

Groups	O		HO		BC		AC		EP	
	P-value	Sig	P-value	Sig	P-value	Sig	P-value	Sig	P-value	Sig
PF&GT	0.000	HS	0.002	S	0.023	S	0.001	S	0.240	NS
PF&PT	0.000	HS	0.000	HS	0.000	HS	0.000	HS	0.000	HS
PF&KO	0.001	S	0.000	HS	0.000	HS	0.000	HS	0.000	HS
GT&PT	0.000	HS	0.000	HS	0.000	HS	0.000	HS	0.000	HS
GT&KO	0.210	NS	0.000	HS	0.000	HS	0.020	S	0.000	HS
PT&KO	0.000	HS	0.000	HS	0.510	NS	0.006	S	0.000	HS

Centering ratio

Table -7 has shown the mean values and the standard deviation of canal centering ratio after instrumentation with the four systems at the five measuring points

Table-7: Mean and standard deviation of canal centering ratio for the four groups at the five levels

System		O	HO	BC	AC	EP
PF	Mean	0.017	0.055	0.018	0.018	0.048
	SD±	0.015	0.012	0.030	0.011	0.016
GT	Mean	0.031	0.057	0.016	0.033	0.034
	SD±	0.003	0.010	0.014	0.015	0.038
PT	Mean	0.329	0.454	0.395	0.377	0.424
	SD±	0.091	0.134	0.117	0.042	0.054
KO	Mean	0.044	0.241	0.476	0.440	0.818
	SD±	0.042	0.068	0.062	0.030	0.037
F-test		163.7	132.1	232.9	103.5	200.0
P-value		0.000 HS	0.000 HS	0.000 HS	0.000 HS	0.000 HS

The lower the score of the centering ratio the better the instruments centered in the canal [6].

At the first level ProFile showed the best centering ability among the other three systems (0.017) and exhibited the worst ability to center in the canal (0.084) at the end point of preparation.GT showed the best centering ability (0.016) at the third level and exhibited the worst ability to center in the canal (0.057) at

the second level.ProTaper showed the best centering ability (0.329) at the first level and exhibited the worst ability to center in the canal (0.454) at the second level.K-flexofile showed the best centering ability (0.044) at the first level and exhibited the worst ability to center in the canal (0.818) at the end point of preparation..

By using the Student t-test (Table -8) a significant difference was found between ProFile and GT at the orifice and not significant at the second, third and fourth levels; while a highly significant difference was found at the last plane. The relation between ProFile and ProTaper was a highly significant difference at all measuring sites; while the variance between ProFile and K-flexofile was significant at the orifice and a highly significant at the other levels.

Also the Student t-test showed a highly significant difference between GT and ProTaper at all levels. The difference between GT and K-flexofile was not significant at the first measuring point; while there was a highly significant difference at the other tested sites. Additionally there was a highly significant difference between ProTaper and K¬flexofile at the first, second and fifth tested points; while there was no significant difference next to the beginning of the curvature (BC). After the curve (AC) the difference between these two groups was significant.

Table-8: t-test of canal centering ratio for the four groups at different levels.

Groups	O		HO		BC		AC		EP	
	P-value	Sig	P-value	Sig	P-value	Sig	P-value	Sig	P-value	Sig
PF&GT	0.024	S	0.680	NS	0.740	NS	0.062	NS	0.000	HS
PF&PT	0.000	HS	0.000	HS	0.000	HS	0.000	HS	0.000	HS
PF&KO	0.014	S	0.000	HS	0.000	HS	0.000	HS	0.000	HS
GT&PT	0.000	HS	0.000	HS	0.000	HS	0.000	HS	0.000	HS
GT&KO	0.220	NS	0.000	HS	0.000	HS	0.000	HS	0.000	HS
PT&KO	0.000	HS	0.000	HS	0.011	S	0.000	HS	0.000	HS

DISCUSSION

Total Canal Diameter

The given results of Table -1 shown that, K-flexofile scored the maximum canal diameter at the apical two levels. This was perhaps as a result of the relatively high rigidity of K-flexofiles if compared with flexible NiTi systems. Stainless-steel instruments tend to straighten when rotating in a curved canal, thus remove more material from the outer walls of the canal. These findings have already noted by Schafer and Schlingemann, 2003b [7]; Perez et al, 2005 [8].

Furthermore, it became obvious that some samples which have been prepared by K-flexofile did not have a continuously tapering form. There were relatively wider regions apical to the beginning of the curve followed by narrow areas towards the orifice. These wider parts near the curve would appear to correspond to the danger zone described by Abou-Rass et al in 1980 [9] where strip perforations occur in vivo.

Comparing NiTi systems, ProTaper prepared the largest canal diameter at all levels and this aggressive behavior of these files has been confirmed by other investigations (Al-Omari et al, 2003 [10]; Bergmans et al, 2003 [11]). This is may be due to three reasons:(a) ProTaper is an active design which has more cutting efficiency if compared with passive instruments like ProFile and GT, (b) the increased taper of ProTaper shaping files of up to 0.19 whereas other instruments were used only with tapers of maximum 0.12 for GT and 0.06 for ProFile, (c) a brushing action that is recommended with this system before further advancing the instruments which may caused, oving more resin coronally.

GT exhibited more dentine removal at the coronal three levels of the canals than ProFile. This finding is coinciding with other studies (Calberson et al, 2002 [5]; Al- Omani et al, 2003 [10]; Bergmans et al, 2003 [11]).This may attribute to the increased taper of the GT up to 0.12 , whereas ProFile is restricted to a 0.06 taper. In contrast, at the apical part of the canals GT performed significantly less canal diameter than Pro-

File. Similar results have been also established by several studies (Garip and Gencoglu [12], 2006; Rodig et al, 2007 [13]). This probably is due to the length of the cutting part of GT is shorter than that of ProFile.

Transportation (outer and inner)

The distance of transportation was determined by measuring the greatest length between the edge of each instrumented canal and the corresponding edge of the un instrumented canal.
Transportation of the canal is determined by the flexibility of the preparation instruments, the movement of the instruments in the canal, as well as the length of time the instrument is in contact with the canal wall during preparation [14].

Concerning the original root curvatures, Tables 3 and 5 reveal that, NiTi systems obtained better canal geometry, demonstrated less canal transportation and straightening at the apical portion of the simulated canals and better maintained the original shape of the curved canals compared with stainless-steel instruments (Fig.3). The direction of transportation observed in this study was generally toward the inner aspect at the coronal and middle parts of the canal and toward the outer aspect of the canal apically. Other studies have confirmed this trend of endodontic instruments (Schafer and Schlingemann, 2003 b [7]; Guelzow et al, 2005[15]).The explanation for this is due to the restoring forces of the instrument in a curved canal which attempt to return the file to its original shape and act on the outer side of the canal wall during preparation and thus lessens its cutting along the inner wall .If this effect is pronounced a significant portion of canal wall remains untouched [16].

In comparison with ProTaper, canals prepared by GT and ProFile maintained original curvature was better with less straightening. ProTaper removed more resin from the outer side at the apical portion of the canal; while it was more efficient on the inner wall at the coronal and middle thirds. These observations are in accordance with recently published studies (Yang et al, 2006 [17]). This fact may be as a results of: (**a**) the sharp cutting edges of ProTaper because of their convex triangular cross- section design [15], (**b**) ProTaper finishing files have progressively tapers resulted in a thicker instrument especially at the apical third of the file cause less flexibility of the instruments when compared with other NiTi systems with the same apical size [18].
In term of outer transportation, Table -3 reveals more cutting effectiveness of GT at the first three levels than ProFile. This may come in agreement with the findings of other studies (Garip and Gencoglu, 2006 [12]; Rodig et al, 2007 [13]). This was as a result of excessive tapering of GT up to 0.12; while it was just 0.06 for ProFile, but this efficiency of GT was decreased by the side of apical two planes .These results were in accordance to a previous reports (Garip and Gencoglu, 2006 [12]; Rodig et al, 2007 [13]). This possibly due to the short cutting part of GT compared with ProFile, which may increase the flexibility of this system.

In regard to inner transportation (Table -5), there were no differences from the results of outer transportation except at the end of preparation where more material has been removed when the canals were instrumented by GT in comparison to ProFile, but the difference was not significant. This may come in agreement with the findings of similar studies (Garip and Gencoglu; 2006 [2]; Rodig et al, 2007 [13]).

Centering ratio

The centering ratio can defined the ability of instruments to remain centered in shaped canals. According to the formula, the centering ratio approaches zero as X1 and X2 become closer .The lower the score, the better the instruments centered in the canal. The flexibility of instruments may be the main factor that allows the instruments to plane the canal walls rather than engaging and screwing into them and to cut of dentin evenly along the canal wall [6].

Table -7 indicated that, the ability of instruments to remain centered in prepared canals was significantly better in NiTi systems than K-flexofiles. Because of the inherent stiffness of stainless-steel instruments there is a tendency to straighten the curved portion of the canal, and consequently this may result in more uneven and excessive dentin removal. By comparing NiTi systems, ProTaper files have excessive tapering this may increase the rigidity of instrument consequently more resin will removed from one side of the canal than the other. Additionally the brushing action which is recommended with this system may cause unevenly resin removal, these factors may explain relatively low centering ability of this system compared with other tested NiTi instruments [19].

Furthermore ProFile were centered in the coronal portion of the canals better than GT, this is related to the increased taper of the GT up to 0.12, whereas ProFile is limited to a 0.06 taper. Excessive tapering may increase the rigidity of instrument consequently more resin will removed from one side of the canal than the other. Conversely GT was better at the end point of preparation. This probably is due to the smooth shank of the GT that may increase the flexibility of this system. These findings confirmed the results previously reported by Park, 2001 [20].

CONCLUSION

K-flexofile scored the maximum canal diameter at the apical two levels. ProTaper prepared the largest canal diameter at all levels. GT exhibited more dentine removal at the coronal three levels of the canals than ProFile. At the apical part of the canals GT performed significantly less canal diameter than ProFile. In comparison with ProTaper, canals prepared by GT and ProFile maintained original curvature was better with less straightening. The ability of instruments to remain centered in prepared canals was significantly better in NiTi systems than K-flexofiles. ProTaper files have low centering ability. ProFile was centered in the coronal portion of the canals better than GT while GT was better at the end point of preparation.

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Root fracture resistance of endodontically treated teeth using three different instrumentation systems (An in vitro study)

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ABSTRACT

Vertical root fractures are result largely from procedures performed in the root canal in the endodontic treatment of roots. In this study three groups of thirty premolars roots have been instrumented with three different systems, hand step back instrumentation with K-files, rotary K3 file system and rotary Protaper file system. The roots then obturated with gutta percha and sealer using lateral condensation technique and then subjected to a vertical load via universal testing machine until fracture. The results showed a statistically significant difference among all groups and a statistically significant difference between group 1 and group 2,3 respectively and no difference between group 2and 3.The hand instrumentation provides more fracture resistance to roots that receive endodontic treatment than the rotary instrumentation techniques.

Introduction

There is a clinical impression that endodontically treated teeth are more friable and fracture easily thus may have to be removed[1].Vertical root fractures are severe complications that are seen in root filled teeth which often lead to extraction[2,3,4,5,6].

A vertical root fracture is a longitudinally oriented fracture of the root extending through the entire thickness of the dentin from the root canal to the periodontium.it may be initiated in the crown or in the root apex or in some cases along theroot between these two points[7].Vertical root fractures are result largely from procedures performed in the root canal in the endodontic treatment of roots for example excessive canal shaping excessive pressure during compaction of gutta percha ....etc.[8]

Lertchirakarnetal 1999 reported fractures resulted from excessive lateral compaction forces during root filling[9], however lateral condensation alone should not be a direct cause of root fracture as loads generated during lateral condensation were significantly lower than the forces required to fracture of roots[10,11].

The instrumentation is un avoidable step in the endodontic therapy thus advancement in the rotary nickel titanium (Ni-Ti) instruments over the last decade have led to a new design concepts and techniques of canal preparation which made the endodontic easier and faster than hand instruments resulting in consistent and predictable root canalshaping[12] in order to create a continuously tapered conical flared preparation advanced instruments designs with non-cutting tips, radial lands , different cross sections, superior resistance to torsional fracture and varying tapers have been developed[13].

Most of the recent systems incorporate instruments with a taper greater than the standard 2%(0.02) and the Ni-Ti instruments available with tapers ranging from 0.04 up to 0.012 and this large taper of these systems may influence the resistance of the endodontically treated teeth roots to fracture.

AIM OF THE STUDY

To compare the fracture resistance of endodontically treated roots using hand standardized instruments technique and other two different rotary Ni-Ti systems the rotary K3 file system and the rotary Protaper system.

Materials and Method:

30 single rooted mandibular premolars will be used.All teeth stored in distilled water until they were tested. teeth will be cleaned with ultrasonic scaler and each tooth will be decoronated at the cement enamel junction with a diamond disc leaving 14mm of each root which will be examined for cracks and defects with magnifying lens and the patency of the canal will be checked by passing no. 10 k-file in the canal until its appear from the apex of the root.

All teeth will be kept moisten in the distilled water throughout the experimental procedure to prevent the dehydration of the roots.

The roots will be divided into three groups:-

1-**Group 1** instrumented by hand step back technique with stainless steel hand k-files.

2-**Group 2** instrumented by crown down technique with Ni-Ti rotary K3-file system.

3-**Group 3** instrumented by crown down technique with Ni-Ti rotary Protaper file system.

Group 1: step back technique with standardized stainless steel hand k- files

The canals will be prepared with hand filling to the master apical file size 30 and then step back with 1mm shorter for the three successive file sizes with recapitulation by the master apical file to the full working length will be performed after each file size of the step back procedure.

Using irrigation solution 2.5% NaOCI after each size preparation by inserting 27-gauge needle. The roots will be stored in distilled water to prevent dehydration.

Group 2: crown down technique with Ni-Ti rotary k3-file system.

The canals will be prepared with k3-files. The Ni-Ti k3-files compromised of 6 Ni-Ti files (two orifice shapers and four shaping files) the instrumentwill be advanced apically with peeking motion until the first sign of resistance detected with a rotation speed between 200-300 rpm.

These instruments are available in different treatment sequences each including six files with size 15-60 with three different tapers(2%,4% and 6%) in addition there are two orifice openers (8% and 10%) for coronal pre flaring. The cross section of the k3-files is asymmetrical(14). Canal preparation will be completed to size 30 with crown down steps using sodium hypochlorite 2.5 % for irrigation.

Group 3: crown down technique with Ni-Ti rotary protaper-file system.

The canals will be prepared with rotary Ni-Ti protaper files at speed 16:1 gear and at 1.4 torques between 250-350 rpm starting with shaper S using multiple passive pressure passes to the working length and later using S2.

Apical part of the canal finished using finishing files F1 and later F2 to the working length. This system have tapers range from (2%,3%,4%,7%,8%,11%,11.5% and 19%) of three shaping files and three finishing files[15].

1-Sx (auxiliary shaper): (iso size 19) taper 3%-19%
2-Sl:(iso size 17) taper 2%-11%
3-S2:(iso size 20) taper 4%-11.5%
4-Fl:(iso size 20) taper 7%-5.5%
5-F2:(iso size 25) taper 8%-5.5%
6-F3:(iso size 30) taper 9%-5%

Canal preparation completed to size 30 using sodium hypochlorite 2.5 % for irrigation.
Recapitulation with size 10 k-file after every instrument used and will be stored in distilled water to prevent dehydration.

Obturation:

After the canals have been dried with absorbent paper points all specimens will be obturated with gutta-percha and zinc oxide based eugenol sealer using lateral condensation technique.

All specimens will be mounted individually in a cold cure acrylic base up to 8mm of the root. The roots will be kept moist using damp towel to prevent dehydration.

Each specimen placed individually on the platform of the Instron testing machine with a round tip that have a 4mm in diameter, this round tip will contact the coronal surface of the specimen and will subject a slowly increase in vertical force of 1mm per minute until fracture occurred when there is a drop in the value of stress applied to the specimen and values will be recorded in Newton then the results will be compared statistically between the different groups.

Results:

The results have been shown a significant difference among the three groups as shown in ANOVA test table.

ANOVA Groups

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	9944.018	2	4972.009	6.201	.006
Within Groups	21649.302	27	801.826		
Total	31593.320	29			

The mean value of fracture point for group 1 was 244 Newton which is higher than the mean value of fracture point for group 2(215).the mean value of fracture point for group3 was 200 Newton which is less than the other two groups as shown in case summaries table.

There is a significant difference between group 1 and group 2as there is a significant difference between group 1 and group 3.

There is no significant difference between group 2 and group 3 as shown in Post Hoc Tests (multiple comparisons) table below

Post Hoc Tests
Multiple Comparisons

Groups LSD

(I) Types	(J) Types	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Stepback	ProTaper	43.9400*	12.6635	.002	17.957	69.923
	K3	28.5700*	12.6635	.032	2.587	54.553
ProTaper	Stepback	-43.9400*	12.6635	.002	-69.923	-17.957
	K3	-15.3700	12.6635	.235	-41.353	10.613
K3	Stepback	-28.5700*	12.6635	.032	-54.553	-2.587
	ProTaper	15.3700	12.6635	.235	-10.613	41.353

*. The mean difference is significant at the 0.05 level.

Discussion:

Root canal instrumentation is an essential stage in endodontic treatment.But it is generally accepted that several endodontic procedures such as access preparation, instrumentation and even irrigation with sodium hypochlorite lead to reduction in fracture resistance of instrumented teeth.

The risk of fractureduring root canal space obturation in both lateral and vertical condensation techniques is high if too much forces exerted during compaction. Studies showed that instrumentation alone has been found to significantly weaken roots. Inasmuch as it is difficult to ascer¬tain the amount of dentine that can be removed before this weakening effect takes place, it seems logical to remove as little dentine as possible during instrumentation without jeopardizing long term success The load required to fracture the root provides an indication of fracture susceptibility of the root when subjected to forces encountered during obturation, post placement, or subsequentclinical function.

When an apical pressure is applied with a round instrument inserted into an elliptical canal, it will bind at its narrowest width, which is typically from mesial to distal. The initial forces will be directed towards the mesiodistal direction leading to a strain on the buccolingual surface. Hence the resulting fracture lines will orient in the buccolingual direction.

Treatment options, destruction of the supporting tissues, opposite to the fracture as a result of the constant release of irritants including bacterial elements to the area, precludes any treatment other than extraction.

The use of CO2 and Nd–YAG laser to fuse fractured roots was tested in an in vitro study, but proved ineffective [16].

The results of this study showed that roots prepared by the hand instruments have higher resistance to fracture than the roots prepared by the rotary systems statistically and this obviously due to the fact that less dentine removal from the inside of the canal when using hand instruments which is due to the design of the instrument itself (taper, cross section) and this agree with the results of ***Shwailiya*** also this finding is agree to the study by ***Wilcox etal***[17],and ***Zandbiglari etal***[18], which concluded that the more root dentin was removed, the more likely a root was to fracture.

Disagree with ***Mirtha etal***[19].which stated that there is more fracture load needed to fracture root prepared with rotary instruments than that prepared with hand instruments this may be a result of the effect of therounder canal shapes preparations leading to reduced areas of stress concentration which may offset the effect of increased dentin removed.

There is no significant statistical difference between fracture loads needed for group 2(K3 file system) and group 3(protaper file system) and this agree with ***Mirthaetal***[19].

There was no variability in the fracture load of the roots, compared to a three-fold range in the ***Lertchirakarnetal***[9]. studyand a four -fold range in the work of ***Pitts etal***[7]. This is presumably because of the variation in root morphology, dentin thickness, calcification, and canal preparation techniques.

Singlaetal.stated that Profile 6% taper instruments offer the advantage of maximum debridement without significant reduction in root fracture resistance compared to step back technique and other tapers of the system**(20)**.

Conclusion:

The hand instrumentation provides more fracture resistance to roots that receive endodontic treatment than the rotary instrumentation techniques. This aspect of endodontic treatment should be considered

in relation with other aspects like ability of cleaning, ability of shaping and ability of fatigue resistance of the instruments.

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The relation between hereditary factor and palatally impacted maxillary canine

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Abstract

Background: it has been well known that there are different etiologies for palatal impaction of maxillary canine ,and one of the most important is the hereditary factor ,but all the previous studies have dealt with the hereditary factor as a presence of ; family history of palatal impaction of maxillary canine , and or missing or peg shaped upper lateral incisor[s}. In this study it was dealt with hereditary factor as if there is a relativity of parents of the patients who have palatally impacted canine.

Materials and methods : the data were collected during a thirty months interval by the examination of 2800 orthodontic patients in Baghdad city and Al-Faluja distinct, 867 male and 1933 female, age ranged between 14 and 29 years .

Results: 44 patients had PIMC, it was found that 63.6% of them had a related parents (cousin marriage), 70.% of them were not presented with clinically diagnosed etiological factors, the majority of these factors were the congenital absence or malformation of maxillary lateral incisor, and the incidence of occurrence were different between male and female .

Conclusion: these observations made us to embark on genetic theory a major role in production of this phenomenon.

Keywords : palatally impacted maxillary canine PIMC, cousin marriage, genetic factor

Introduction :

malocclusion is a manifestation of genetic and environmental interaction on the development of orofacial region . ,orthodontist may be interested in genetics to help understand why a patient has a particular occlusion.(1)

Impacted teeth are those with a delayed eruption time or that are not expected to erupt completely based on clinical and radio graphical assessment (2). apart from the third molar, the maxillary canine is the most frequently involved tooth with abnormal developmental position (3,4,5) , due to denser palatal bone and thicker palatal mucosa , as well as amore horizontal position ,palatally displaced cuspids rarely erupt without requiring complex orthodontic treatment.(6)

The prevalence of palatally impacted maxillary canine is low , but it seems to have a variable distribution with regard to ethnic origin (7) ,the incidence of impacted maxillary canine is around 2-3% (8,9,10) ,whereas Dachi and Howell (11) reported that the incidence is about 0.92% , and they occur more commonly in females than males in the ratio of 2:1.

Etiology: the exact etiology of palatally impacted maxillary cuspid is un known; however, 2 common theories may explain the phenomenon : the guidance theory and the genetic theory(12,13). The guidance theory of palatal canine displacement proposes that this anomaly is a result of local predisposing causes including congenitally missing lateral incisors , supernumerary teeth , odontomas , transposition of teeth and other mechanical determinants that all interfere with the path of eruption of the canine (2,14,15,16),maxillary canine develop high in the maxilla ,are among the last teeth to develop and travel along path before they erupt into the dental arch (17).

these factors increase the potential for mechanical disturbances resulting in displacement and , thus , impaction.(13)

the second theory : focuses on genetic causes for impacted cuspids (12,13),given the strong hereditary influence in palatal canine displacement , there are those who believe that this is the principal factor involved and dismiss other relationships as secondary or as similarly linked hereditary factors, in another words , the palatal canine is another link in the chain of genetically linked phenomena (7).palatally impacted maxillary cuspids often presents with other dental abnormalities including tooth size , shape ,number, and structure which Baccetti (18) reported to be linked genetically .

In a study of the families of children affected by palatally displaced maxillary canines , a search was made on the parents and the siblings for the related anomalies , The prevalence of small, peg shaped and missing lateral incisors, late developing dentitions and other missing teeth among these close relatives was very high, in addition to palatally impacted canines ,This evidence points to heredity as the causal agent for these associated phenomena(10), We have contended that their presence creates an environment favorable to the development of palatally displaced canine (18)

Other factors : crowding; Hichin { 1956}[16] considered that crowding of the dentition was the reason for this condition , although he offered no evidence to support his contention .in general, crowding of the dentition results in the exaggerated displacement of a tooth from its developmental position in the arch [7]
Trauma:in a recent clinical report Brin et al [1993b] [15] have illustrated how trauma , which leads to a cessation in the development of a lateral incisor root , may be associated palatal canine impaction.

Non- resorption of the root of the deciduous canine : this usually results in mild displacement of the permanent tooth buccally .However, if the permanent canine itself is displaced, normal resorption of the deciduous tooth will not occur ,in this situation the retained deciduous tooth is an indicator , rather than the cause , of displacement [14]

Materials and methods

the data were collected during a thirty months interval by the examination of 2800 orthodontic patients in three orthodontic clinics in Baghdad city and Al-Faluja distinct, 867 male and 1933 female, age ranged between 14 and 29 . For every patient diagnosed preliminarily to have one or two PIMC(s) [non eruption of canine beyond its normal time of eruption] the followings were done :

1-*clinical examination* by inspection and palpation to reveal the followings:

a- bulging of the soft tissue at the area of impaction to give primary diagnosis about the direction of the impacted canine .
b-unilateral or bilateral missing or peg shaped maxillary lateral incisor(s)
c-presence of supernumerary teeth or transposition of teeth.
d-presence of crowding

2-*radiographic examination* which include two types of radiographs:

a-orthopantamograph(OPG):for the primary assessment of the position of the impacted canine, diagnosis of supernumerary teeth, confirm diagnosis of maxillary lateral incisor missing and diagnosis of any abnormality or odontoma.
b-periapical radiograph: A parallax technique were used to localize the impacted canine(if it is impacted palatally or buccally), by using two periapical radiographs and a horizontal tube shift [19] .

-Patients *history* :
1- the parents were asked if they are related (cousin marriage) or not by detailed questions about the relation (they should belong to the same grand father or mother).
2- the patients were asked about the presence of previous history of trauma , patients with history of previous trauma were excluded .

Results:
The total number of patients had PIMC was 44(32 in Al-Faluja ,12 in Baghdad), these patients arranged in 4 tables considering the following variables; gender, parents relation, and clinically found etiological factors.

Table(1) represents the distribution of patients with PIMC according to parents relation and frequency of etiological factors:

Total number of patients with PIMC(44)	Patients with not related parents Group2		Patients with related parents Group1	
	Not presented with etiological factors	presented with etiological factors	Not presented with etiological factors	presented with etiological factors
	10	6	21	7

Table (2) grouping of percents of patients according to parents relation and presence of etiological factors:

Group1: patients with related parents	63.6%
Group2: patients with not related parents	36.3%
Group3: patients presented with etiological factor	29.5%
Group4: patients not presented with etiological factor	70.4%

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Table(3) represents the distribution of occurrence between males and females and frequency of occurrence(unilateral or bilateral):

Total number of patients with PIMC(44)	Female(35)		Male(9)	
	Bilateral	unilateral	Bilateral	Unilateral
	6	29	1	8

Table(4) distribution according to the type of etiological factors in G1and G2

Total number of patients (44)[#]	Missing lateral incisors	Peg shaped lateral incisors	Crowding	Super -numerary teeth	Transposition
Group 1	2.2%	2.2%	0%	4.5%	4.5%
Group 2	2.2%	2.2%	0%	4.5%	4.5%

[#] no odontoma was found in all patients.

Discussion :

the displacement of any tooth in the dental arch is usually related to the presence of a single or multiple obstructive factors interfering with normal development and eruption , for the impacted maxillary canine ; a new character is added which is the loss of guidance represented by the absence of maxillary lateral incisor , even though , many cases of impacted canine are not associated with one of these factors . on a parallel line , the researches was found that in some cases the impaction occur in more than one member of the same family in association , some times ,with malformed or missing teeth, which turned the light on the genetic factor as being a major factor in the occurrence of this abnormality ,and for this reason this study was conducted .

By comparison of numbers and percents of group 1 with group 2 ,we notice that the rate of occurrence is higher in group 1,tables (1and2) which indicates the presence of something different from the known causes of palatal canine impaction(like the loss of guidance ,space adequacy, and long path of eruption) . This high rate of occurrence in the group of related parents might support the theory of genetic origin of PIMC [10,13,15,19] , and it can be explained as the appearance of some recessive hereditary features in the siblings of parents who are related(cousin marriage) [cousin marriage has genetic aspects that do not arise in the case of other marriage –related political and social issues like interracial marriage . this is because married couples possessing higher than normal consanguinity have ,on average, an increased chance of sharing genes for recessive traits] [20]. So that for this difference in the frequency of occurrence between the two groups ,we can say that it may be a phenotype that appear more frequently in relatives(consanguinity can cause unmasking a hidden recessive gene , mating between relatives , or inbreeding causes an increase in the frequency of homozygote's among the offspring , recessive phenotype appear with greater frequency among the progeny of inbreed mating than in the general population [21].

on the other hand, the difference between groups 3 and 4 indicates the greater possibility of occurrence in the absence of obvious clinical predisposing factors, which means, there is a hidden factor that cause the problem , which is most probably genetic in nature .

beside that ,we can notice that there is a gender difference in the prevalence of PIMC,(table 3), in which male to female ratio was 1:4, and there is a difference in the frequency of bilateral occurrence ,male to female ratio was 1:6, this difference might be an indicator to a hereditary problem occur in female more than male.

The findings of this study also indicate that, the PIMC may be a separated phenomenon since 79.3% of the cases of PIMC are not associated with missing or peg shaped lateral incisors, which differ from that of Brin I et al 1986 [6] who found that 47.7% of patients with palatally impacted canine have small ,peg-shaped or missing lateral incisors ,but in the same time, we can notice that the higher percents of predisposing factors

were occupied by the missing or peg shaped lateral incisors which are basically blamed to be genetically determined .table(4)

Finally ,from the results of this study ,it was found that the percent of patients with PIMC who have enough space was 97.6% , which agree with the findings of ,becker 1984 [7]and Brin et al1986][15] who pointed out that the likelihood of palatally impacted canine is much reduced were crowding is present.

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Relationship between periodontal diseases and C-reactive protein among hypertensive patients under β-blocker antihypertensive drug

(Clinical and Biochemical study)

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ABSTRACT

Background: Hypertension is the most important public health problem in the world and one of the major risk factors for cardiovascular diseases, and it has been reported that hypertension is linked with periodontal diseases and both condition have reported to elevated levels of C-reactive protein.

Aims of the study: To determine the periodontal health status and the concentration of C-reactive protein in saliva among patients with hypertension and under β-blocker treatment and to compare the results with systemic healthy individuals, also to correlate the clinical findings with biochemical findings.

Materials and Methods: Test group consist of 25 hypertensive patients and under β-blocker treatment{Atenolol(Tenormin) 50 mg/day}, the test group further subdivided into three groups according to the duration of medication into: test I group under medication less than one year, test II group under medication between one to four years, test III group under medication more than four years.In addition to25 control group. Their age was between (40-45) years and all patients in both groups were male and non-smokers. Periodontal disease was evaluated by recording the plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment level. Unstimulated salivary samples were collected and then chemically analyzed using high sensitivity ELISA to determine the concentration of C-reactive protein.

Results: The mean value of all recorded periodontal parameters were highest among test group compared to control with statistically significant difference existed between both groups(*p=0.001*) for plaque index, (*p=0.008*) for gingival index, (*p=0.006*) for bleeding on probing, (*p=0.017*) for probing pocket depth, (*p=0.002*) for clinical attachment level.

In regard to the concentration of salivary C-reactive protein, the mean value was highest among test group compared to control with statistically non-significant differences between both groups(*p=0.606*).

The correlation coefficient between salivary C-reactive protein and periodontal parameters showed statistically non-significant correlation in both test group and control. Among test group, result revealed statistically significant correlation between salivary C-reactive protein and pocket depth among test group III (under medication more than four years)(*p=0.039*).

Conclusion: The study revealed poor condition of the oral cavities regarding the periodontal condition of patients with hypertension, so the co-operation between general practitioners, cardiologists and dentists needs to be intensified. The concentrationsof salivary C-reactive protein of test groups were higher than control.

Key words: Periodontal diseases, hypertension, C-reactive protein

INTRODUCTION

Periodontium is a complex and highly specialized pressure-sensing system consisting of four tissues (cementum, periodontal ligament, alveolar bone, and junctional and sulcular epithelia) supporting the teeth. Of these structures, periodontal ligament is a dynamic tissue with a high rate of remodeling and turnover, which connects the teeth to the alveolar bone [(1)]. Periodontal disease is defined as a chronic inflammatory disease of teeth-supporting tissues. The spreading of the inflammatory process from the gingiva deep into periodontium tissues may lead to the destruction of the alveolodental ligament and considerable bone loss in the alveolar process. Although the primary cause of periodontitis is bacterial infection, several systemic diseases seem to be associated with the development of destructive periodontal disease [(2)].

Hypertension is one of the most important risk factors for strok, myocardial infarction, peripheral vascular disease, heart failure, and end-stage renal disease. Hypertension is a major global public health problem. It is affects approximately one billion people worldwide and estimated that about 26.4% of global adult population have hypertension with two third of them living in economically developing nations [(3-4-5)].Despite the general agreement that arterial hypertension is a significant risk factor for cardiovascular diseases. Recent evidence have linked periodontal diseases with high blood pressure [(6-7)]. Other measures of poor oral health (tooth loss) have been associated with hypertension [(8)].The rational for an association between hypertension and periodontal disease is based on findings showing that hypertension leads to morphological changes of vessels feeding the periodontal membrane, affecting the position of the tooth [(9)].

Saliva is very important body fluid often taken for

granted. It is critical to the maintenance of oral health. Saliva has also become useful as a non-invasive alternative for blood in medical diagnosis and research [10].

C-reactive protein (CRP) is a non-specific inflammatory marker present in the blood and saliva and commonly used in paediatrics in the diagnosis and monitoring of inflammation and active infection, as C-reactive protein values increase markedly during these acute processes. C-reactive protein elevated in hypertension, and elevated among those with periodontal diseases [11-12-13]. High sensitivity methods have recently been developed and permit the determination of CRP levels far below those found in inflammatory processes [14].

MATERIALS AND METHODS

sample

Study group consisted of (25 males, non-smokers) with confirmed diagnosis of hypertension under medication (which include only those patients under β-blocker {Atenolol(Tenormin) 50 mg / day}. Patients under other types of medication and/or with any other systemic disease were excluded from this study. Their age was ranged between (40-45 years). A control group of (25 males, non-smokers and without any systemic diseases) matching with age of study group was also examined.

1-Periodontal clinical examination

Periodontal clinical parameters were carried out for all permanent teeth and four surfaces of each tooth were scored by using mouth mirror, diagnostic probe, and periodontal probes. The following indices were recorded:

A.Plaque index [15]

By using dental mirror and diagnostic probe and before salivary sample collection, the criteria for this index:

Score 0: Absence of plaque deposits

Score 1: Plaque disclosed after running the periodontal probe along the gingival margin.

Score 2: Moderate accumulation of plaque not exceeding one third of tooth surface and can be detected by naked eye.

Score 3: Abundance amount of plaque exceeding one third of tooth surface.

B.Gingival index [16]

The criteria of this index are:

Score 0: Entire absence of visual signs of inflammation in the gingival unit.

Score 1: Mild inflammation, slight change in colour, slight edema, no bleeding on probing.

Score 2: Moderate inflammation, moderate glazing, redness, edema and hypertrophy, bleeding on probing.

Score 3: Sever inflammation, marked redness and hypertrophy ulceration, tendency to spontaneous bleeding.

C.Bleeding on Probing

The four surfaces of each permanent teeth were evaluated by running the periodontal probe gently inside the gingival sulcus, If bleeding occurs within 30 seconds after probing, the site was given a positive score, and a negative score is given for non-bleeding sites
.

D.Probing Pocket Depth

The distance from the gingival margin to the location of the tip of a periodontal probe inserted in the pocket with moderate probing force was recorded by at four surfaces of all examined permanent teeth.

E. Clinical Attachment Level (*Ramfjord 1959*)

The distance from the cemento-enamel junction (CEJ) to the location of the inserted probe tip was measured at four surfaces of all examined teeth. Loss of attachment was done according to Ramfjord by:

- Measuring the distance from the free gingival margin to the bottom of the gingival sulcus .The interproximal recording done at the buccal aspect of the inter proximal contact.
- Measuring the distance from the free gingival margin to the cemento-enamel junction.
- The attachment loss was obtained from subtracting the above two Measurement.
- Loss of attachment in case of gum recession was recorded by adding the first measurement to the second one.

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2-Biochemical analysis

Whole saliva was collected by tilting the head forward, allowing the saliva to pool on the floor of the mouth, then passing the saliva into a disposable test tube, Samples visibly contaminated with blood were recollected. Sample collection within 60 minutes after eating a major meal was avoided because acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth and to minimize these factors, rinse mouth thoroughly with water before sample was collected. After collection the samples keep cold, in order to avoid bacterial growth and loss of CRP in the specimen and all samples were freezed below -20ºC as soon as possible after collection. Freezed saliva samples will precipitate the mucins. On day of assay, thaw completely and centrifuge at 3000 rpm for 15 minutes then subjected to biochemical analysis to determine the concentration of C-reactive protein using ELISA .

RESULTS

Results of salivary C-reactive protein are illustrated in Table (1).The application of Student's t–test for salivary C-reactive protein revealed a statistically non-significant difference between the test group and control group.

The comparsion of periodontal parameters between the control group and test group has been illustrated in Table (2). The Student's t–test was used and results revealed a statistically significant differences of all periodontal parameter(PlI, GI, BOP, PPD, CAL) between the control group and the test group.

The correlation coefficient between CRP with PlI, GI, BOP, PPD, and CAL, was clarify in Table (3). these results revealed statistically non-significant correlation for both groups. Table (4) demonstrates the correlation coefficient between C-reactive protein with PlI, GI, BOP, PPD, and CAL among the test groups. Results showed a statistically significant correlation of CRP with PPD among test group

DISCUSSION

The mean concentration of C-reactive protein was higher in the saliva of the test group than the control group the explanation of this result was due to that hypertension increase the level of salivary C-reactive protein, this result agree with previous stud which shown that C-reactive protein is positively associated with hypertension [17-18]. For periodontal parameters, the mean for plaque index in test group was higher than the control group The possible explanation of such results may be due to that people with hypertension neglect the oral hygiene measures and did not brush their teeth regularly.This result agree with previous study [19]. Results of the GI and BOP in the present study showed that mean of these indices were higher among test group than control, and a statistically significant differences between the test group and control group This result coincid with previous study [20]. Regarding the PPD and CAL, the present study clarified that the mean of these parameters were higher in test group than control group and results illustrated significant differences between them, these finding are in concordant with many studies which has shown that high blood pressure was significantly associated with the prevalence of 5 millimetres or deeper periodontal pockets, and loss of attachment [21-22]., Suggested potential mechanisms linking periodontal inflammation with BP may be refered to a direct effect on endothelial dysfunction or an indirect stimulation of a systemic metabolic/inflammatory host response. For correlation coefficient of salivary C-reactive protein with periodontal parameters, it was only significantly correlated to pocket depth ($p < 0.039$)in test group III, this may be due to the increase in surface area for bacteria, plaque, and calculus to inflict a chronic infection, while the results of correlation coefficient between salivary C-reactive protein and the remaining periodontal parameters in both test and control groups were non- significant. These finding were supported by [13-23] who demonstrated a positive correlation between C-reactive protein and severity of periodontal disease.

Table 1:

Control Group		Test Group		t–value	*p*–value
Mean	+ SD	Mean	+ SD		
1867.7948	1307.7668	2084.1978	1624.2753	–0.519	0.606

Table 2:

Parameters	Control Group		Test Group		t–value	p–value
	Mean	+ SD	Mean	+ SD		
PII	0.77552	0.529025	1.30608	0.560560	–3.442	0.001*
GI	0.66104	0.508803	1.09744	0.603545	–2.764	0.008*
BOP	0.14528	0.212398	0.37268	0.330381	–2.895	0.006*
PPD(mm)	3.75444	1.436294	4.48116	0.302877	–2.475	0.017*
CAL(mm)	2.17204	0.720295	2.70228	0.367945	–3.278	0.002*

Table 3:

Parameter	CRP (pg/ml)			
	Control Group		Test Group	
	r–value	*p*–value	r–value	*p*–value
PII	0.102	0.626	0.300	0.145
GI	0.132	0.528	0.271	0.189
BOP	–0.020	0.924	0.323	0.115
PPD (mm)	–0.031	0.884	–0.063	0.764
CAL (mm)	–0.194	0.354	0.223	0.283

Table 4:

Parameter	CRP (pg/ml)					
	Test I Group		Test II Group		Test III Group	
	r–value	*p*–value	r–value	*p*–value	r–value	*p*–value
PII	0.243	0.562	–0.579	0.133	0.626	0.071
GI	0.438	0.278	–0.566	0.144	0.536	0.137
BOP	0.337	0.414	–0.473	0.236	0.598	0.089
PPD (mm)	–0.477	0.232	–0.582	0.130	0.692	0.039*
CAL(mm)	0.273	0.513	–0.200	0.635	0.444	0.232

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Evaluation of Interleukine-1β (IL-1β) levels in Plasma of Patients with Periodontal Diseases and their correlation with Body Mass Index

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Abstract

Background and objectives: Obesity is emerging as a significant health problem worldwide and risk factor for various systemic diseases. Periodontal disease is multifactorial inflammatory disease. Recent evidence points to a link between body mass index and periodontitis, and role of Interleukin-1β (IL-1 β) has been suggested.

Materials and methods: One hundred and sixty subjects were divided into two groups: 80 healthy subjects as a control group and 80 patients with periodontitis. Their age ranged from 25 to 45 years. Then subjects of both groups were subdivided in to four subgroups based on the WHO classification of Body Mass Index(BMI): group A; Under-weight (BMI< 18.5kg/m^2), group B; Normal-weight (BMI 18.5-24.95 kg/m^2), group C; Over-weight (BMI 25-29.95kg/m^2) and group D; Obese (BMI >30 5kg/m^2). Full-mouth periodontal assessment was performed and Clinical periodontal parameters included clinical attachment level (CAL) and probing pocket depth (PPD). The quantitative assay of IL-1β was performed in serum, using an ELISA method. The BMI, IL-1β levels in plasma and periodontal Disease Index (PDI) scores were assessed, compared and correlated.

Results: In patients with periodontitis, significantly higher clinical periodontal parameters (CAL and PPD) and IL-1β levels were found in obese subgroup as compared to other subgroups. Also, a significant and positive correlation was seen between BMI and IL-1β, CAL and PPD, while correlation coefficient between mean scores of PPD, CAL and mean concentration of IL-1β in sera were statistically highly significant and negative respectively. In control subgroups, a highly significant increased level of IL-1β was observed in sera of obese subgroup when compared to other subgroups.

Conclusion: Increase in the level of IL-1 β in sera and an increase in the severity of periodontitis seen in subjects with higher body mass index. This may indicate that obesity may be detrimental to the periodontal health of individuals.
Key words: Periodontitis; BMI, Interleukine-1β (IL-1β).

INTRODUCTION

Periodontitis, is a chronic inflammatory disease of the periodontium occurring in response to bacterial plaque on the adjacent teeth; characterized by destruction of the alveolar bone and periodontal ligament, apical migration of the epithelial attachment resulting in the formation of periodontal pockets, and ultimately loosening and exfoliation of the teeth [(1)].
Obesity is a complex multifactorial chronic disease that develops from an interaction of genotype and the environment among obese subgroups. Obesity has a significant association with periodontitis in terms of body mass index (BMI). The BMI has always been considered a simple method for analysis of the nutritional status. These findings suggest that periodontitis may be aggravated by certain conditions associated with obesity for example, "the metabolic syndrome", a clustering of dyslipidaemia and insulin resistance [(2)].

It is now clear that adipose tissue is complex and metabolically active. It secrets numerous immunomodulatory factors and plays a major role in regulating metabolic and vascular biology. Adipose cells secrete more than 50 bioactive molecules, known collectively as adipokines which include Interleukine-1Beta (IL-1β), which may inhance periodontal degradation [(3)].

IL-1β is a pro-inflammatory cytokine that plays a pivotal role in several chronic diseases produced by monocyte, macrophage, and epithelial cells[(4)].This cytokine is a primary activator of early chemotactic cytokines, as well as of the expression of adhesion molecules that facilitate migration of leucocytes in to tissues. IL-1β is also known to be one of the most active stimulators of osteoclastic bone reabsorption [(5)].

IL- 1β stimulates a variety of cell types to produce connective tissue catabolic and bone-resorptive mediators, including IL-6, TNF-α, prostaglandin E2, and matrix metalloproteinase [(6)] .These factors lead to the degradation of connective tissue such as collagen, along with the recruitment and activation of osteoclasts. Much attention has been given to the influence of IL-1β on bone turnover, particularly in pathologic disease processes such as periodontitis [(7)]. IL-1β has been shown to have a dual function in collagen digestion. It inhibits the intracellular phagocytic pathway, but at the same time strongly promotes extracellular

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digestion by inducing the release of collagenolytic enzymes such as collagenase [8].

Data indicate that increased body mass index, serum adipokine levels and percentage of subcutaneous body fat are associated with increased risk for periodontitis. For instance, more bleeding on probing, deeper periodontal pockets and more bone loss were noticed in individuals with higher indicators of obesity [9].

Additionally, increased amounts of adipokines (e.g.1L-1β, TNF-α, Leptin) from visceral fat may induce agglutination of blood in the microvasculature, decreasing blood flow to the gingiva in obese people and facilitating the progression of periodontitis. Despite the accumulating evidence for significant associations, it is still unclear whether obesity truly precedes periodontitis. However, maintaining a normal body weight, eating a well-balanced diet and engaging in physical activity have been shown to reduce the severity of periodontitis [10].

Periodontal disease is no longer identified as only an oral health problem but also a public health issue as it is associated with systemic health. Many mediators have been recognized for this relationship like chronic inflammation, infection and genetic predisposition. Apart from these mediators, nutrition has been postulated as an alternative mediator[11]. High body mass index, the most common nutritional disorder, is a significant risk factor for numerous adult diseases, and may be a factor in the incidence of periodontitis [12].

The link between BMI and periodontal disease may not be completely understood, but it is clear that once established, this relationship will prove to be of extreme public health relevance. It may go a long way in planning and modifying preventive and treatment modalities for periodontal disease. Hence this study was planned to evaluate the relationship between BMI, 1L-1β, and periodontal disease.

MATERIALS AND METHODS

A cross-sectional study on association between periodontitis with BMI in Erbil city province was carried out. Study subjects were recruited from department of periodontology, College of dentistry, Hawler Medical University in Erbil city. The data was collected during the period of 10th January up to 17th May 2011.

A total One hundred and sixty subjects have been included in this study, 80 healthy subjects that represented a control group and 80 patients with periodontitis that represented the study group. Their age ranged from 25 to 45 years. They were selectively included during their visit to the department of periodontics. Then subjects of both groups were subdivided in to four subgroups based on the WHO classification of Body Mass Index: group A; Under-weight, group B; Normal-weight; group C; Over-weight and group D; Obese. All patients were systemically healthy. This study involved height and weight measurements for determination of BMI, done by ourself and another assistant. A structured questionnaire was completed by each subject. Dental examinations were carried out by experienced periodontist. Blood samples were collected after an overnight fasting. Those selected were categorized into 4 subgroups, as summarized in (Table 1). Ethical approval was obtained from the College of Dentistry/Hawler medical university in Erbil city. Informed consent obtained from participants who were classified as under-weight, normal weight, over-weight, and obese according WHO classification. Inclusion and Exclusion criteria are presented in (Table 2).

Table (1): Flow table illustrated the number of groups and subgroups of control and patient with periodontal condition associated with BMI.

Groups / Sub-groups	Periodontitis N=80	Control N=80	BMI(kg/m²)
A	20 Under-weight	20 Under-weight	< 18.5
B	20 Normal weight	20 Normal weight	18.5 - 24.9
C	20 Over-weigh	20 Over-weight	25 - 29.9
D	20 Obese	20 Obese	≥ 30

Table (2): Flow table illustrated Inclusion criteria and Exclusion criteria

Inclusion criteria	Exclusion criteria
Age (25-45).	Current alcohol and smokers
Systemically healthy.	Prior antibiotic used one week before the study
Clinical attachment loss ≥ 4mm	Prior use of systemic corticosteroids within the last month
Number of teeth ≥ 10 teeth	General dental scaling in the last month
	Systemic diseases. e.g. DM, hypertension, CVD
	Current medications influencing the periodontal tissues within the last month.
	Current pregnancy

Probing pocket depth (PPD): The distance from the gingival margin to most apical extent of Williams probe inserted in to gingival crevice as close as possible to the long axis of tooth at four surfaces of each tooth was recorded in millimeter (mm) [13]. The sites for measurement were mesio-buccal, mid buccal, disto-buccal, mid-lingual lines. No pressure was used the probe was allowed to fall by its own weight.

Clinical attachment loss (CAL): This was assessed by measuring the distance from the cement-enamel junction (CEJ) to the base of the pocket [13], the level of CEJ could be determined detecting it with a probe [14], when CEJ was obliterated by the gingival margin, the CAL was measured indirectly by subtracting the distance in millimeters from the gingival margin to the CEJ from PPD at each site. In cases when there was a gingival recession, loss of attachment was measured by adding the distance from the gingival margin to the CEJ to PPD at each site [15]. The criteria for periodontitis in the present study was defined by patient who had two or more interproximal sites with CAL of 4mm or more (not in the same tooth) non djacent teeth and (PPD ≥ 4mm) [16].

Body mass index (BMI): was used to indicate overall adiposity (kg/m^2). It was calculated using each participant's weight in (Kgs) divided by the (m^2).

$$BMI = \frac{\text{Weight in (Kgs)}}{\text{Height in (m}^2\text{)}}$$

The recommended WHO Standard BMI classification was applied in this study.

Blood Samples and Processing

Blood samples were collected after an overnight fasting. Venous blood samples were obtained from enrolled subgroups by vein puncture five milliliter was withdrawn from each patient and control subgroups. Five ml were collected into sterile plain tubes; the blood was left for a while at room temperature to clot. Then it was centrifuged at 2500 rpm for 10 min, the serum was separated and transferred into sterile screw capped labeled tubes and stored at – 20C°. The stored plasma was used for estimation of IL-1β levels at a later date using an enzyme immunoassay for the in vitro determination of IL-1β in plasma. The assay was carried out as per manufacturer's directions for use by the commercial **KOMA BIOTECH INC** IL-1β ELISA kit (**Seol , Kores**).

Statistical analysis

The collected data were analyzed by using Computer program software SPSS (Statistical Package for Social Sciences); version **15** (www.spss.com).

1- Descriptive statistics which include: Mean; Standard deviation; Tables, Multiple Dot Diagram and Column chart.

2- Inferential statistics which included:

a- Post-Hoc Comparisons to see exactly which groups are significantly different.

b- T-test used for statistically significant difference between group mean was tested.

c- The Pearson correlation coefficient was used to analyze linear relationship of each two variables. Results were considered significant, if P value ≤ 0.05.

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RESULTS

For the clinical periodontal parameters, from (Table 3) showed a descriptive statistics of the mean scores and standard deviation of periodontal parameters (CAL and PPD) for four periodontitis subgroups (under weight, normal, over and obese weight), it has been shown that the mean of CAL for obese subgroup was highest one which was (5.70 ± 0.62), than Under, Normal, Over weight subgroups which were (4.49 ± 0.16, 4.78 ± 0.30, 5.17 ± 0.52) respectively, and also PPD for obese subgroup was the highest one which was (4.35 ± 0.38), when compared with Under, Normal, Over weight which were (4.0 ± 0.16, 4.10 ± 0.21, 4.20 ± 0.34) respectively. Indicated that the mean scores of CAL and PPD in obese subgroup was greater and decreased with decreasing in BMI. Figure (1 and 2) showed mean and standard deviation of clinical periodontal parameters (CAL and PPD) in each periodontitis subgroups with total mean and standard deviation for all periodontitis subgroups together in relation to BMI.

Regarding the periodontitis subgroups, the mean concentration of IL-1β in obese subgroup was (7.83pg/mL) while declined in over-weight, normal weight and under-weight were (5.46, 4.16, 3.078 pg/mL) respectively. Regarding control subgroups, the same table showed a marked increase in mean concentration of IL-1β in obese subgroup (3.45 pg/mL) when compared with under weight, normal and over weigh control subgroups which were (2.57, 3.32 and , 3.31, pg/mL) respectively

Results obtained from (Table 4), showed that the mean concentration of IL-1β, was higher in high BMI category than in low BMI category in both periodontitis and control groups, and high in periodontitis group than control group, the same findings were more clear in (**Figure 3**).

By using Post Hoc Test as demonstrated in (Table 5), indicated that there were a highly significant difference in mean scores of CAL between obese weight and overweight ($p<0.001$), however, there were non significance difference in mean scores of PPD between this two subgroups ($p>0.05$). As shown from the same table there were highly significant differences in mean scores of CAL and PPD between obese and normal weight ($p<0.001$, $p<0.01$) respectively. While, there were highly significant difference in mean scores of CAL and PPD between obese weight and under weight ($P<0.001$, $P<0.01$) respectively. Whereas, highly significant differences in serum IL-1β level between obese weight and normal weight ($P<0.001$). Similarly, highly significant difference in serum IL-1β, was observed between obese weight and under weight ($P<0.001$).

Results illustrated in (Table 6), by using Post Hoc Test showed that statistically highly significant differences in serum IL-1β levels ($P<0.001$) between obese weight and under weight. The same data showed a highly significant differences in serum IL-1β levels between obese weight and normal weight, obese weight and over weight ($P<0.001$ and $P<0.001$) respectively.

By Pearson correlation coefficient analysis, for obese weight periodontitis subgroup, analysis the correlation between the mean scores of PPD and mean scores of CAL was ($r=0.594^{**}$, $p<0.05$) were highly significant and positive. Correlation coefficient between mean scores of PPD, CAL and mean concentration of IL-1β in sera ($r=-0.694^{**}$, -0.644^{**}, $P< 0.01$) were statistically highly significant and nnnnegative, respectively (Table 7).

Table 3: The descriptive statistics for the clinical periodontal parameter (CAL and PPD) in periodontitis subgroups in relation to BMI.

Variables	types of weight	N	Mean	Std. D.	Std. E.
CAL(mm)	Under-weight	20	4.49	0.16	0.362
	Normal weight	20	4.78	0.30	0.067
	Over-weight	20	5.17	0.52	0.115
	Obese	20	5.70	0.62	0.139
	Total	80	5.03	0.63	0.070
PPD(mm)	Under-weight	20	4.00	0.16	0.036
	Normal weight	20	4.10	0.21	0.045
	Over-weight	20	4.20	0.34	0.076
	Obese	20	4.35	0.38	0.084
	Total	80	4.16	0.309	0.035

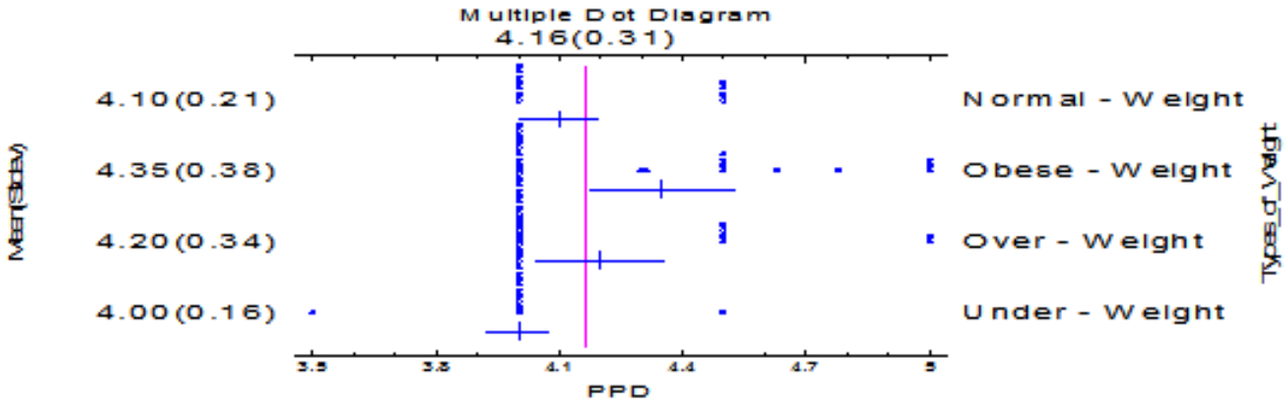

Figure 1: Multiple Dot Diagram demonstrated mean scores and standard deviation of periodontal parameters (PPD) in each sub-group with total mean and standard deviation in all periodontitis subgroups together in relation to BMI.

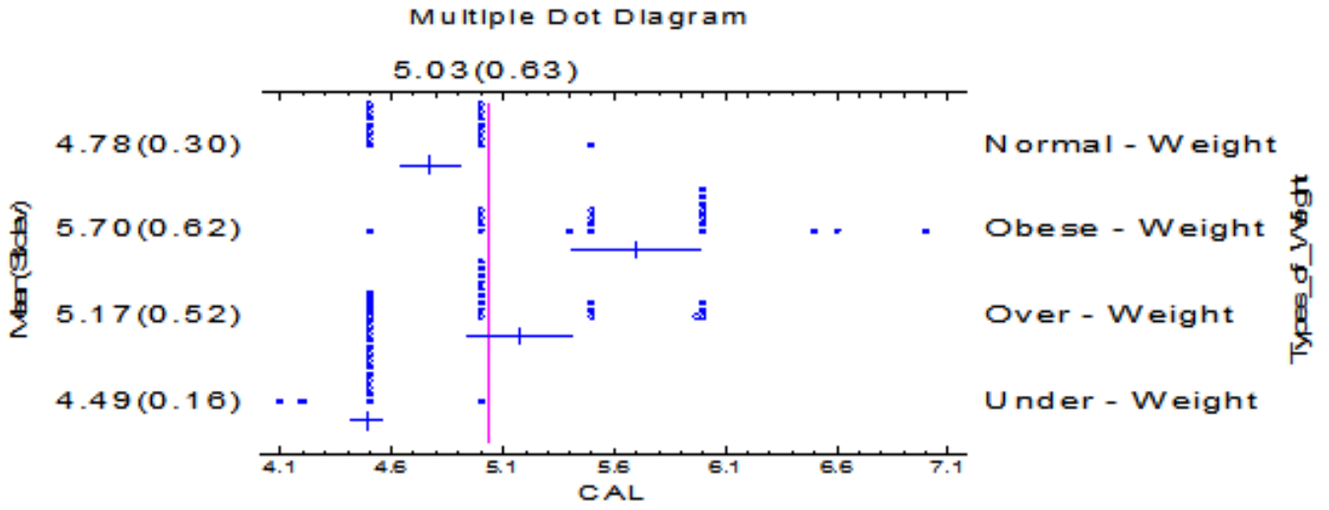

Figure 2: Multiple Dot Diagram demonstrated mean scores and standard .deviation of periodontal parameters (CAL) in each subgroup with total mean and standard deviation in all periodontitis subgroups together in relation to BMI.

Table (4): The descriptive statistics for the mean concentration of serum IL-1β (pg/ml) in each of periodontitis and control groups according to BMI.

Serum cytokine	Groups	Under-weight Mean ± S.D.	Normal-Weight Mean ± S.D.	Over-Weight Mean ± S.D.	Obese Mean ± S.D.	Total N=160 Mean ± S.D.
IL-1β (pg/ml)	Periodontitis Subgroups N=20	3.08 ± 0.82	4.16± 1.24	5.46± 0.96	7.83± 1.67	5.13± 2.15
	Control Sub-groups N=20	2.57± 0.36	3.32±0.95	3.31±0.89	3.45 ± 1.07	3.14± 0.91

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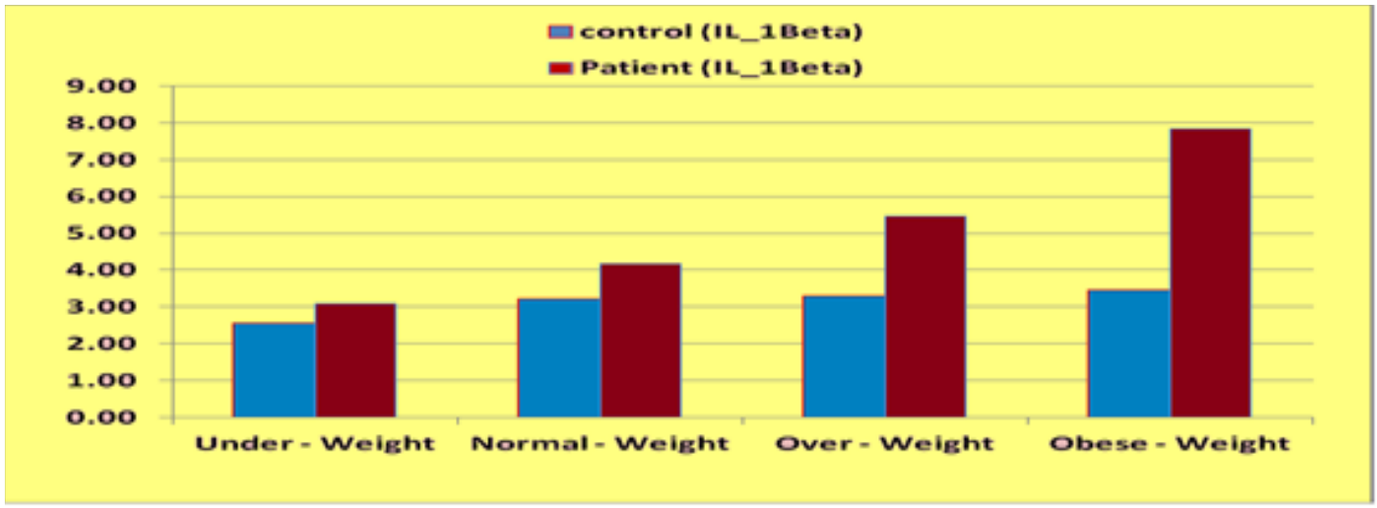

Figure 3 : Column chart for detection of mean concentration of IL-1β (pg/mL) in sera for both periodontitis and control subgroups in relation to BMI.

Table 5: Comparison of Obese-weight periodontitis subgroup with other periodontitis subgroups (Under-weight, Normal-weight, Over-weight) by Post Hoc Test.

Dependent Variable	(I) type of weight (kg/m^2)	(I) type of weight (kg/m^2)	Mean difference (I-J)	Std. Error	Sig.
PPD (mm)	Obese	Under-weight	0.351	0.091	0.004**
		Normal-weight	0.251	0.091	0.007**
		Over-weight	0.151	0.091	0.099^N
CAL(mm)	Obese	Under-weight	1.210	0.139	0.000**
		Normal weight	0.925	0.139	0.000**
		Over-weight	0.527	0.139	0.000**
IL-1β (pg/ml)	Obese	Under-weight	4.756	0.385	0.000**
		Normal weight	3.672	0.385	0.000**
		Over-weight	2.372	0.385	0.000**

Table 6: Comparison of Obese-weight control subgroup with other control subgroups (Under-weight, Over-weight, Obese weight) by Post Hoc Test.

Dependent Variable	(I) type of weight (kg/m^2)	(J) type of weight (kg/m^2)	Mean difference (I-J)	Std. Error	Sig.
IL-1β (pg/ml)	Obese	Under-weight	0.873	0.273	0.002**
		Normal-weight	0.215	0.273	0.434N
		Over-weight	0.133	0.273	0.629N

Table 7: Correlation between periodontal scores (CAL and PPD) and serum IL-1β level in obese weight periodontitis subgroup.

Variables	PPD (mm)	CAL (mm)	IL-1β (pg/ml)
PPD Pearson correlation	1	0.595**	-0.694**
Sig. (2-tail)		0.006	0.001
N	20	20	20
CAL person correlation	0.595**	1	-0.644**
Sig. (2-tail)	0.006		0.002
N	20	20	20
IL-1β Pearson correlation	-0.694**	-0.644**	1
Sig. (2-tail)	0.001	0.002	
N	20	20	20

DISCUSSION

It has been suggested that obesity contributes to an overall systemic inflammatory state through its effect on metabolic and immune parameters, thereby increasing susceptibility to periodontal disease [17]. In recent years, the evidence linking obesity to increased incidence and severity of periodontal disease has grown [18].

Increasingly, evidence of a relationship of newly identified risk factors for systematic diseases to periodontal disease is starting to emerge. In this light, recent studies indicate that obesity is emerging as a risk indicator for periodontal disease [19]. In this study, the correlation between serum IL-1β level and periodontal disease in relation to BMI has been evaluated. Studies have shown that obese subjects have abundant cytokines in the serum due to cytokine release from adipose tissue [20]. It may be speculated that the raised serum cytokines are transported at higher levels in to the gingival tissue [21] .

The periodontal parameters in present study were clinical attachment loss and probing pocket depth. The present findings showed a statistically significant correlation between BMI and periodontal measurements of CAL, and PPD. The severity of both clinical attachment loss (CAL) and probing pocket depth (PPD) was significantly associated to BMI. This study confirms that the severity of CAL and PPD, specially the severity of CAL was higher in both obese and over-weight subgroups than other two subgroups which were normal and under-weight.

In this study, increased in the severity of CAL and PPD with increasing in BMI corresponds with the work by team workers; they founded that increased BMI were found to be significantly associated with the presence of CAL and PPD, [18].

Our findings are similar to those of cross-sectional study by [9], found that there is a significant correlations between BMI and mean of CAL and PPD. Ylöstalo and coworkers (2008) [22] reported a strong association between BMI and the presence of pockets. Moreover,[23] concluded overweight and obese individuals that might put them at risk for initiation and progression of periodontitis and risk was significantly higher in obese individuals.

The relationship between BMI and periodontal variables was showed by [24] were in contrast to the results of present study was significantly no associated present between CAL, PPD and BMI,

Increase in BMI associated with periodontitis, high BMI has been postulated to reduce blood flow to the periodontal tissues, promoting the development of periodontal disease[25]. Furthermore, high BMI may enhance immunological or inflammatory disorders, which might be the reason for obese subjects, tend to exhibit escalating poor periodontal status relative to non-obese individuals [19]. Furthermore, it has been shown that adipose tissue secretes several proinflammatory cytokines, also implicated in periodontitis[24]. As host response to local bacterial challenge is a key factor in determining periodontitis susceptibility, an increased inflammatory state as that found in obese individuals could predispose them to increased periodontal tissue destruction [27].

The present study demonstrated that periodontitis was significantly associated with serum

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level of IL-1β when compared with control subject, and also a highly statistically significant elevation of serum concentration of IL-1β were observed in those with the highest quartile of BMI compared to those in the lowest quartile because obesity is likely to result in greater IL-1β production. A study was done by Boch and co-workers (2001)[28] corroborated above mentioned finding of IL-1β levels as a biomarker of periodontal disease. Elevated levels IL-1β was found to significantly increase the risk of periodontal disease. The levels of IL-1β could serve as biomarkers of periodontitis. IL-1β levels have been reported to be elevated in periodontal tissues in high BMI when compared to low BMI. A study done by Gamonal and colleagues (2003)[29] similar to our results showed that the evaluation of the IL-1β was performed comparing periodontal patients and individuals without disease. There was a significant difference in IL-1β when comparing the high BMI subgroup with the low subgroup. Ziccardi and co-workers (2002)[30] stated that obese and over-weight individuals have raised levels of circulating IL-β compared with those of normal weight, with some reduction in cytokine levels on weight reduction, this is agree our results and with Saxlin and co-workers (2009b)[31] suggested that there was a significant association between periodontitis and an association between serum IL-l-β, body weight and periodontitis.

In contrast to our findings Hodge and co-workers (2001)[32] and Tai and colleageus (2002) [33],who observed no significant differences in the serum IL-1β levels between periodontitis, control subgroup and BMI in European and in Japanese individuals, respectively. This was due to their study design, which was recruited higher range of age and their definition to periodontitis was different from the definition used in this study

In obese periodontitis subgroup, Pearson correlation coefficients of CAL and PPD with obesity were determined in present study. The results demonstrated a highly significant positive correlation ($P< 0.01$). The results of our study were similar to those reported by, (Saito and Shimazaki, 2007) [34], they found a highly significant positive correlation between obesity and periodontitis. Whereas highly negative correlation were found between CAL, PPD and IL-1β ($r=-0.694^{**}$, -0.644^{**}, $P< 0.01$), disagree with results of, (Ikezawa-Suzuki et al., 2008)[35], this may due to the design of their study and definition of periodontitis

Our results confirm those reported by[36] whom concluded that severity of periodontal disease could be evaluated through IL-1β activity. Identified IL-1β as playing a pivotal role in the pathogenic mechanism of periodontal tissue destruction. According to the authors, clinical parameters such as probing depth (PD). Moreover, the degree of inflammation within periodontal disease tissue could be measured IL-β activity in diseased tissues based on the classification of clinical parameters.

CONCLUSION

Clinical attachment and probing pocket depth appear to be highly significant in obese subgroup when compared with other subgroups. Serum IL-1β level appear to be high in case of periodontitis than control subgroups. Our study strengthens the idea of higher of proinflammatory cytokine IL-1β concentrations in sera in high BMI subgroup relative to low BMI subgroups. This indicated that obesity may be detrimental to the periodontal health of individuals.

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Evaluation of Addition of Plant Fixed Oil Extracts (Flax, Rosemary) as Antifungal on Color Stability of a Heat Cured Soft Lining Material

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ABSTRACT

Introduction: Loss of softness, increase hardness and colonization of acrylic soft liner denture base material by C. albicans are significantly inevitable clinical problems, therefore enhancement of softness and giving antifungal activity to soft liner material to lengthen the life span of it are recommended.

Aims: This study were conducted to evaluate the addition of plant fixed oils extract (flax and /or Rosemary) as antifungal and their effect on color stability of heat cured acrylic soft liner material cured by two different cycles.

Materials and Methods: Total number of (112) samples have been prepared in this study. Samples were divided into two main groups, according to the method of curing into short and long cycles. Each main group has been sub divided into four sub-groups, according to the type of additive materials { Flax, Rosemary, mixed (Flax and Rosemary), and control group}.

Color stability and antifungal tests were applied on each of two main groups (short and long cycle). The color stability test was done at two different periods of immersion in distilled water (two and thirty days); except for antifungal tests were done after (two, seven and thirty) days of immersion in distilled water.

Results: The results showed that acceptable levels of color change (ΔE) in vitro were obtained after oils addition to soft liner (vertex). The soft liner with oil showed antifungal activity to some extent.

Conclusions: Conclusions revealed that the addition of Flax oil and Rosemary oil showed antifungal activity to some extent, with no effect on the acceptable range of the physical properties (color).

Introduction:

Denture soft lining materials are usually used in prosthodontics to provide a cushioning layer on the fitting surface of a complete denture. The material absorbs some of the masticatory energy and reduces the energy transmitted to the underlying tissues [1].

One of the more serious problems is the colonization of soft liner surface by C. albicans [2].The adherence of C. albicans to polymers such as denture acrylic resins or soft lining materials is the first step in colonization, yielding to the development of pathogenesis and eventually causing infection [3].

Acrylic resin liners are frequently affected by water sorption and a loss of chemical components, which can alter their stiffness [4]. The success of the soft lining materials depends partly on their color stability, and softness upon time [5].

A resilient liner with antifungal activity can be of great advantage for patients with a high risk of denture stomatitis [6]. Candida can readily colonize and actually invade soft denture liner. Particularly, in the case for patients whose denture cleansing regimens are poor. In such instances antifungal agents may be considered as an adjunct in the management strategy, therefore, there have been attempts to incorporate antifungal agents directly into the soft liner material [7].

Plants extracts have traditionally been used in folk medicine, as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts [8]. Oils are concentrated, hydrophobic liquid containing volatile aromatic compounds from plants. They possess a wide spectrum of pharmacological activities. The antimicrobial effects of oils have been documented and used in herbal medicine in many countries [9].

MATERIALS AND METHODS

Short and long Cycles Groups:

Fifty six samples cured by short curing cycle according to (ADA Specification No. 12, 2009)[10], samples cured for 90 minutes in 70°C followed by 30 minutes in 100°C, and fifty six samples cured by long curing cycle (9 hours in 73°C) using thermostatically controlled curing unit. The color test was done after two days and thirty days intervals and for antifungal tests that were done at 2, 7, 30, day's intervals respectively. These groups were sub divided into four sub-groups according to the type of additive materials. The sub groups as follows:

a.Flax oil as additive group (FS), and (FL).
b.Rosemary oil as additive group (RS), and (RL).
c.Equal mixture of Flax and Rosemary oils as additive group (FRS), and (FRL)..
d.Control without additives group (CS), and (CL).

Preparation of the samples:

Dental flasks with dental stone (Elite, Zhermack SPA, Bovazecchino, Italy) as investment material was used with the hard plastic foils (Imprelon, Scheu Dental- GmbH) of different thicknesses and shapes

(ADA Specification No. 12, 2009) using computerized numerical control machine to produce molds of production of different samples used in the study. For color stability a model of 30 mm length, 15mm width and 3mm thickness, while for antifungal tests, a disc prepared according to International Standards Organization [11], 5 mm in diameter were aseptically fabricated. The surface area of each specimen was 55 mm2 [12,13].

Denture soft lining material (Vertex) samples have been prepared according to manufacturer instruction with powder: liquid ratio of 3gm: 2.5ml. For samples with oils addition, the plant oil extracts (Flax and Rosemary) were mixed with monomer (14) at concentration of 5% per volume by an adjustable micropipette (DragonLab, China) with a ratio of 125µL for each 2.5ml monomer.

After complete curing, the samples were removed from their moulds, and stored in containers with non-ionized distilled water for each specific test at 37°C temperature for two days, and thirty days immersion time until tests were performed.

Color Stability:

Sample's shape and size was described previously, at two time intervals; two days and 30 day after curing samples and immersion in non ionized distilled water, sample of all groups were assessed for its color stability. A digital image scanner (Epson, China) was used for scanning and obtaining samples images, each sample was scanned at 600dpi, and the resultant jpg image used for color assessment by image processing tool of Matlab 2010 software to obtain L*a*b values for each sample image.

A special syntax script was programmed and added to image processing tool in order to export the L*a*b* values for all pixel of the image, and then the average L*a*b* values were recorded automatically into Microsoft Excel file. The L*a*b* values for each sample were calculated two times after two days (L1*a1*b*1), and after thirty days (L2*a2*b*2). The (CIE L*a*b*) color difference metrics were used for the color stability analysis of the samples in this study. The total color change (ΔE) of each sample was calculated. For each sample for color evaluation using the equation below:

$$\Delta E = [(L^*2-L^*1)2 + (a^*2-a^*1)2 + (b^*2-b^*1)2]1/2$$ [15].

If (ΔE=0) no color difference was detected after exposing the sample to the testing environment. A (ΔE=3.7) or less was considered to be clinically acceptable in vitro, while (ΔE=6.8) was considered to be clinically acceptable in vivo study [16, 17].

Antifungal Tests:

To evaluate antifungal activity of extracted oils (Flax and Rosemary), and antifungal potentials of modified denture soft lining materials after addition of oils, two different tests were used. *C. albicans*, the target fungus, was isolated and incubated for testing purposes.

***C. albicans* Isolation and Identification:**

C. albicans was obtained by taking swabs from ten volunteers wearing old dentures attending Department of Prosthodonotics at College of Dentistry / University of Mosul. The collected swabs cultured on Sabouraud's dextrose agar and incubated at 37°C for 24 hours.

To identify C. albicans after incubation and to select the pathogenic strain according to prototype ATTC strain of *C. albicans* (No. 10231, American Type Tissue Culture) from the collected ten swabs, the following tests were done:

1. Culture morphological features assessment for *C. albicans* colonies, it should be creamy to white, flat or domed, and have a dry glistering or waxy surface [18].
2. *C. albicans* takes gram positive stain. It appears under light microscope as spherical to oval budding cells (3-6 nm) in the yeast or the blastospore form [19].
3. [18,20]showed the ability of pathogenic *C. albicans* to form germ tube, by incubating a loop full Candida in 0.5 ml serum for 3 hours at 37°C. A wet film reveals the presence of filamentous out growth, germ tube from which *C. albicans* can be readily differentiated from other species.
4. The isolated fungus was also identified by *C. albicans* API kit which is a standardized system for Candida species identification to ensure that the isolated fungi met the ATTC 10231 strain for C. albicans (American Type Tissue Culture No. 10231,).

Disc Diffusion Susceptibility Test of C. albicans for Different Antifungal Agents and Oil Extracts:

Candida albicans susceptibility to different antifungal agents compared with oil extracts (Flax and/or Rosemary) was investigated using disc diffusion method. A sterile filter paper discs Wattman No.1 was prepared and immersed in 15:l of two antifungal solutions (Nystatin 100000 IU and Fluconazole 3mg/ml). Another set (two set for Flax and Rosemary) of filter paper discs were prepared and immersed into 15:l of Flax oil and Rosemary oil respectively [21].

A loopfull from fresh *C. albicans* culture was taken and inoculated in 5 ml of nutrient broth and incubated for 24 hours at 37°C, a swab from the broth was cultured on Mulluer Hinton agar, one disk of each antifungal solutions and two oil extracts (Flax and/or Rosemary) discs (one disc for each) were placed on the surface of the culture as shown in figure (1), and then the culture was incubated for 24 hours at 37°C, Five duplicates were done for each disc. The inhibition zones were measured using ruler, the average values then calculated [22, 23].

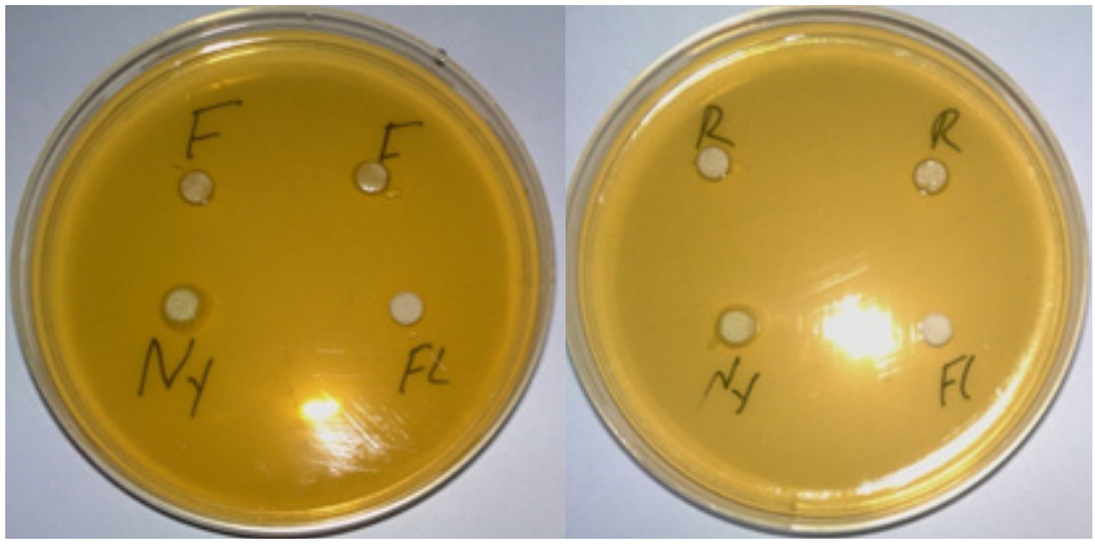

Figure (1). Disc diffusion Susceptibility tests of Candida albicans, A: Flax oils and control. B: Rosemary oil and control.

Modified Denture Soft Lining Material Antifungal Activity Assessment by Standard Colony Counting Test:

Samples from each sub-group for both short and long curing cycles were investigated for its antifungal potentials using standard colony counting test. Modified denture soft lining material samples with additives and control without additives were prepared according to (ISO, 1992) standards with total surface area of 55mm2, as it described previously. This test was repeated over three periods: two, seven, and thirty days after curing. *C. albicans* broth culture was prepared by taking a loop-full from fresh *C. albicans* culture then inoculated into 5 ml of nutrient broth and incubated for 24 hrs at 37°C, then compare with tube of McFarland No. 3.

Each of described disc specimens were immersed in a test tube containing 4.5 ml of a sterilized nutrient broth then it inoculated with 0.5 ml of *C. albicans* broth culture of McFarland No. 3 (as mentioned above), another 24 hours incubation was done at 37°C for the test tubes containing disc specimens. After incubation, the broth was removed with a sterile pipette. The specimen discs were rinsed 5 times with sterile distilled water to remove the loosely adherent *C. albicans*. Then the discs were carefully placed in sterile test tubes that contained sterile saline and placed over digital electric shaker (DragonLab, China) for 60 minutes. Dilution by taking 0.5ml from the supernatant added to 4.5ml of nutrient broth, and then 100µL of each diluted supernatant was placed by using glass spreader on Petri dish plates that contained Sabouraud's dextrose agar. The plates were returned to the incubator at 37°C for 24 hours. Colony formation was then counted after incubation [12, 13]. This method is accepted by ATTC 10231 (American Type Tissue Culture) and it was implemented by many authors: [24, 21, 23,13, 25].

Statistical Analysis:

Statistical analysis was made using SPSS 19 computer software; One way analysis of variance ANOVA followed by Duncan's multiple range tests were used to compare between groups.

Results and discussion:

Color Stability of Soft Lining Material:

L*A*B* Means and Standard Deviation:

The color stability of four different groups of soft liner cured by short cycle and long cycle after storing in distilled water at different periods of time (two and thirty days) was evaluated. Means and standard deviation of (L*A*B*) values for the four groups of short cycle at two and thirty days periods were shown in figure (2). While for long cycle at two and thirty days periods were shown in figure (3).

Measuring Color Changes According to the (CIE L*A*B*) Color system:

To measure color changes of denture soft lining ma-

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terials, color change (ΔE) comparison between short cycle sub-groups (control group with other sub-groups). All sub-groups of short cycle group showed an accepted (ΔE) value in vitro after modification of denture soft lining materials, as shown in table (1) for two days and thirty days period

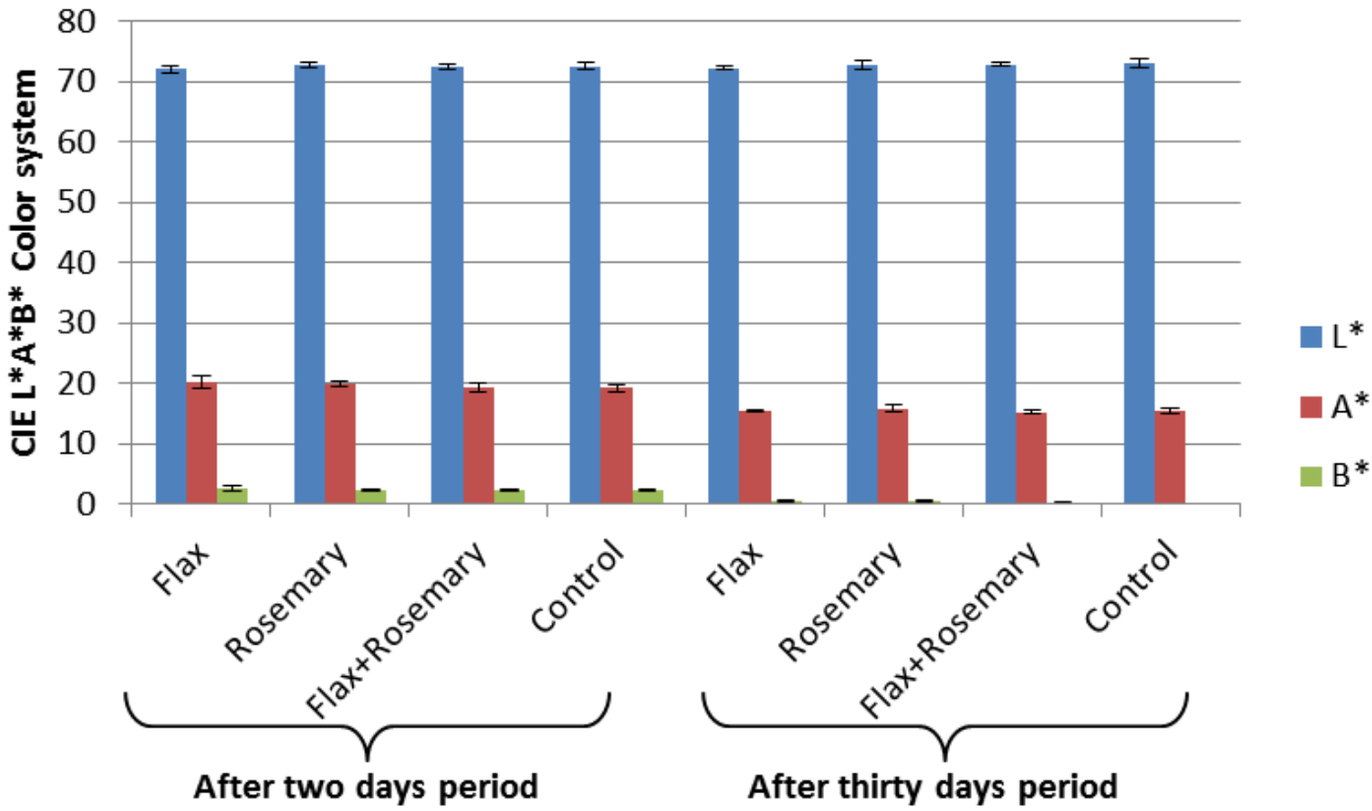

Figure (2). Mean and ± standard deviation for (L*A*B*) values for short cycle groups at two and at thirty days periods.

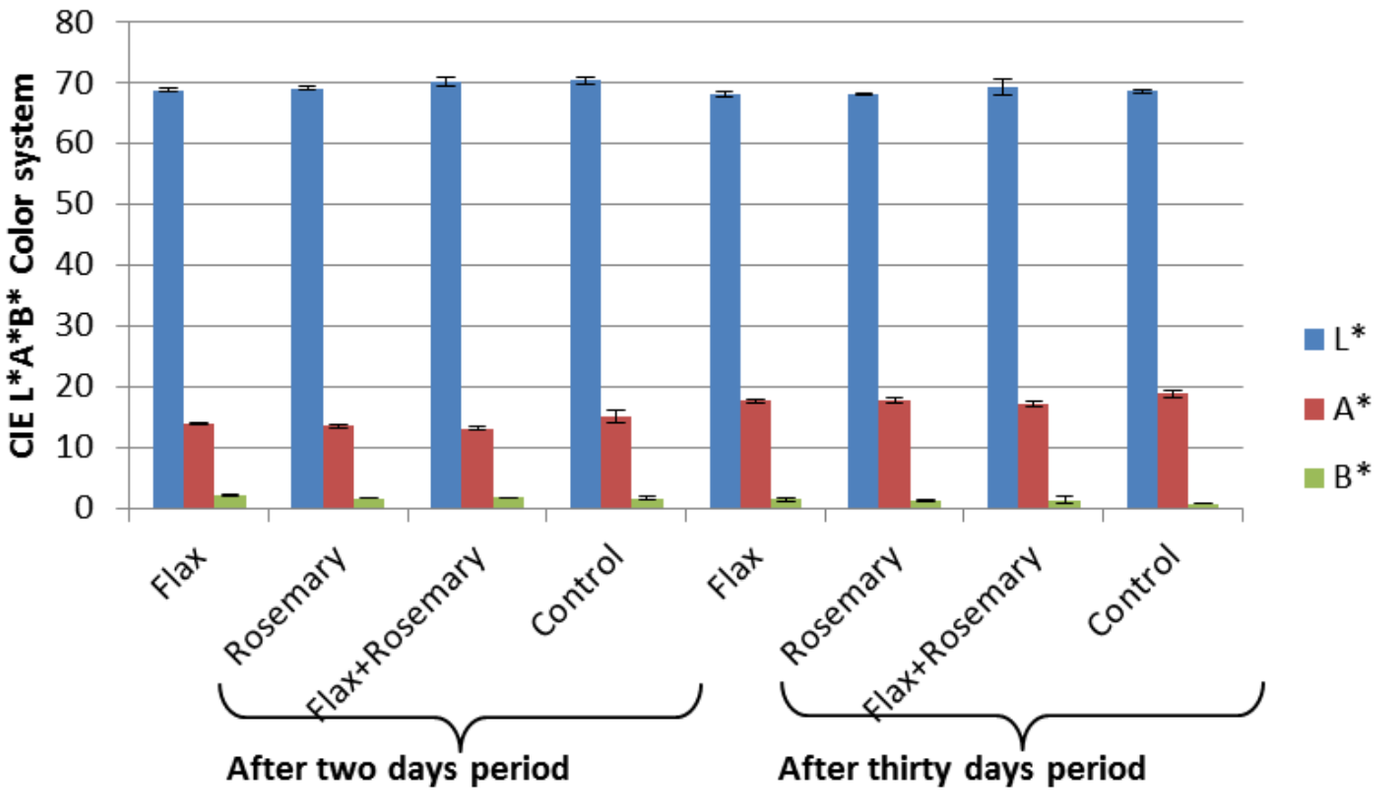

Figure (3). Mean and ± standard deviation for (L*A*B*) values for long cycle groups at two and at thirty days periods.

For long cycle groups, a comparison between its sub-groups (control group with other sub-groups) was made. All sub-groups of long cycle group showed an accepted (ΔE) value in vitro after modification of denture soft lining materials, as shown in table (1) for two days and thirty days period.

Table (1). Color changes in (CIE L*A*B*) color system between short cycle and long cycle groups at two, and thirty days period.

Sub-group	ΔE	In vitro	ΔE	In vitro
	Short cycle(Two days)		Short cycle(30 days)	
FS vs CS	1.120577	Acceptable	0.840081	Acceptable
RS vs CS	0.678493	Acceptable	0.553109	Acceptable
FRS vs CS	0.152886	Acceptable	0.2986	Acceptable
	Long cycle(Two days)		Long cycle(30 days)	
FL vs CL	1.899686	Acceptable	1.440598	Acceptable
RL vs CL	1.942214	Acceptable	1.362461	Acceptable
FRL vs CL	1.869191	Acceptable	2.014417	Acceptable

(ΔE) = 0 no color change; (ΔE) ≤ 3.7 accepted in vitro color change.

To find color changes after immersion of samples for thirty days another comparison were done between same sub-group at two days period and thirty days periods. All sub-groups for both short and long cycle groups were shown an in vitro not acceptable color changes (even for control group); while it showed an acceptable color changes in vivo. Short cycle and long cycle color changes described in table (2).

Table (2). Color changes in (CIE L*A*B*) color system for short and long cycle groups comparing between two days and thirty days periods.

Sub-group	ΔE	In vitro	In vivo	Sub-group	ΔE	In vitro	In vivo
	Short cycle				Long cycle		
FS vs FS	5.177563	Not acceptable	Acceptable	FL vs FL	3.873428	Not acceptable	Acceptable
RS vs RS	4.511494	Not acceptable	Acceptable	RL vs RL	4.376261	Not acceptable	Acceptable
FRS vs FRS	4.578319	Not acceptable	Acceptable	FRL vs FRL	4.063143	Not acceptable	Acceptable
CS vs CS	4.353215	Not acceptable	Acceptable	CL vs CL	4.321842	Not acceptable	Acceptable

(ΔE) = 0 no color change; (ΔE) ≤ 3.7 accepted in vitro color change; (ΔE) ≤ 6.8 accepted in vivo color change.

Antifungal Activity Results:

Disc Diffusion Test Results: *C. albicans* susceptibility to different antifungal agents (Nytatin and Fluconazole) compared with oil extracts (Flax and Rosemary) was investigated using disk diffusion method on Mulluer Hinton agar. Four groups (flax oil, rosemary oil, Nystatin, Fluconazole; respectively) were subjected to disc diffusion test each group with five duplicates. Inhibition zones were shown in figure (4). One way ANOVA test comparing the means of inhibition zones for each group was shown in table (3); there were significant differences between groups at $p \leq 0.05$.

Table (3). One way ANOVA, test for disc diffusion antifungal activity.

	Summation of Squares	Degree of freedom	Mean Square	F	Significance
Between Groups	923.800	3	307.933	68.430	0.000
Within Groups	72.000	16	4.500		
Total	995.800	19			

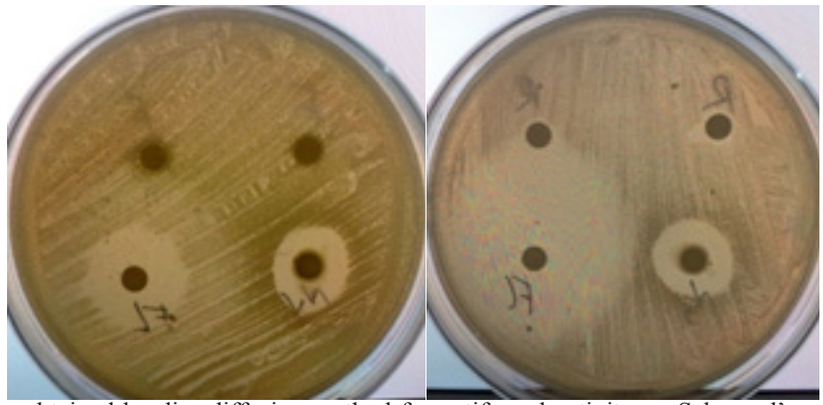

Figure (4) Inhibition zones obtained by disc diffusion method for antifungal activity on Sabaroud's agar media. A. for Flax oil compared with Fluconazole and Nystatin, B. for Rosemary oil compared with Fluconazole and Nystatin.

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Inhibition zone means measured in (mm) with its standard deviation and Duncan's multiple range are shown in figure (5) showing significant differences between groups.

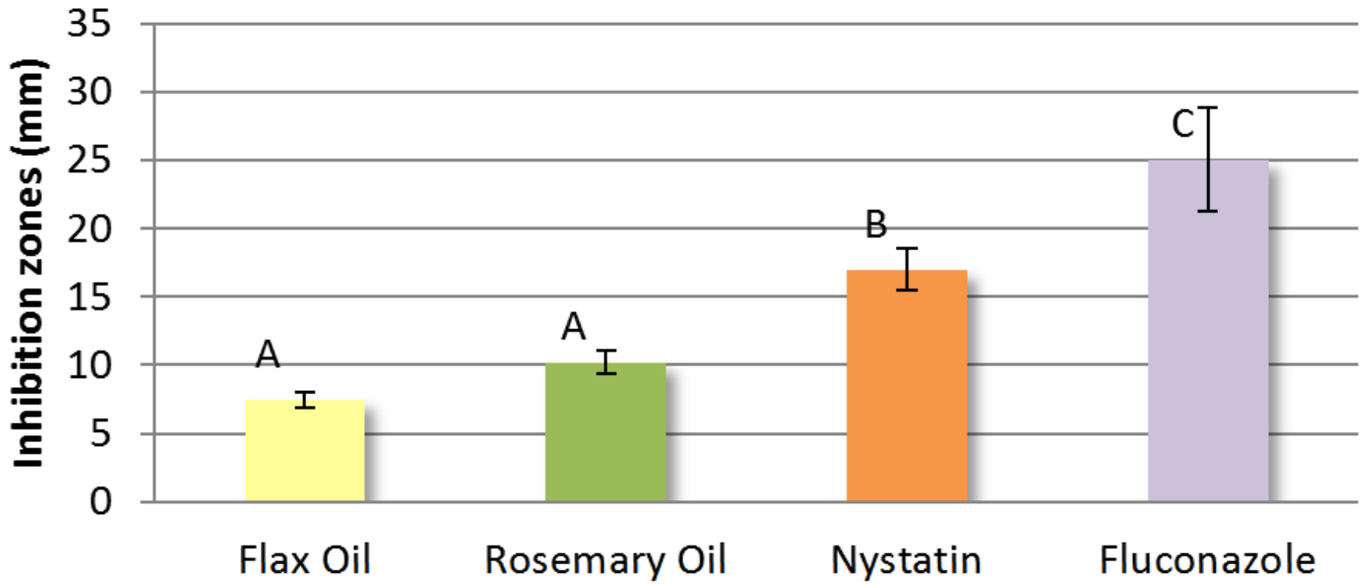

Figure (5). Inhibition zone means in (mm) for disc diffusion antifungal test.
Different letters means significant differences for Duncan's multiple range tests.

Standard Colony Count Test Results:

Denture soft lining materials samples from each sub-group for both short and long curing cycles were investigated for its antifungal potentials using standard colony counting test. Standard colony tests were repeated for two, seven, and thirty days for both short and long cycle.

(Figure 6 and 7) showed trend line for colony counts for both short and long cycle sub-groups over the three mentioned period.

Short cycle sub-groups results for two; seven and thirty days were shown in figure (8), (9) and (10) respectively.

Long cycle sub-groups for two, seven and thirty days were shown in figure (11), (12) and (13) respectively.

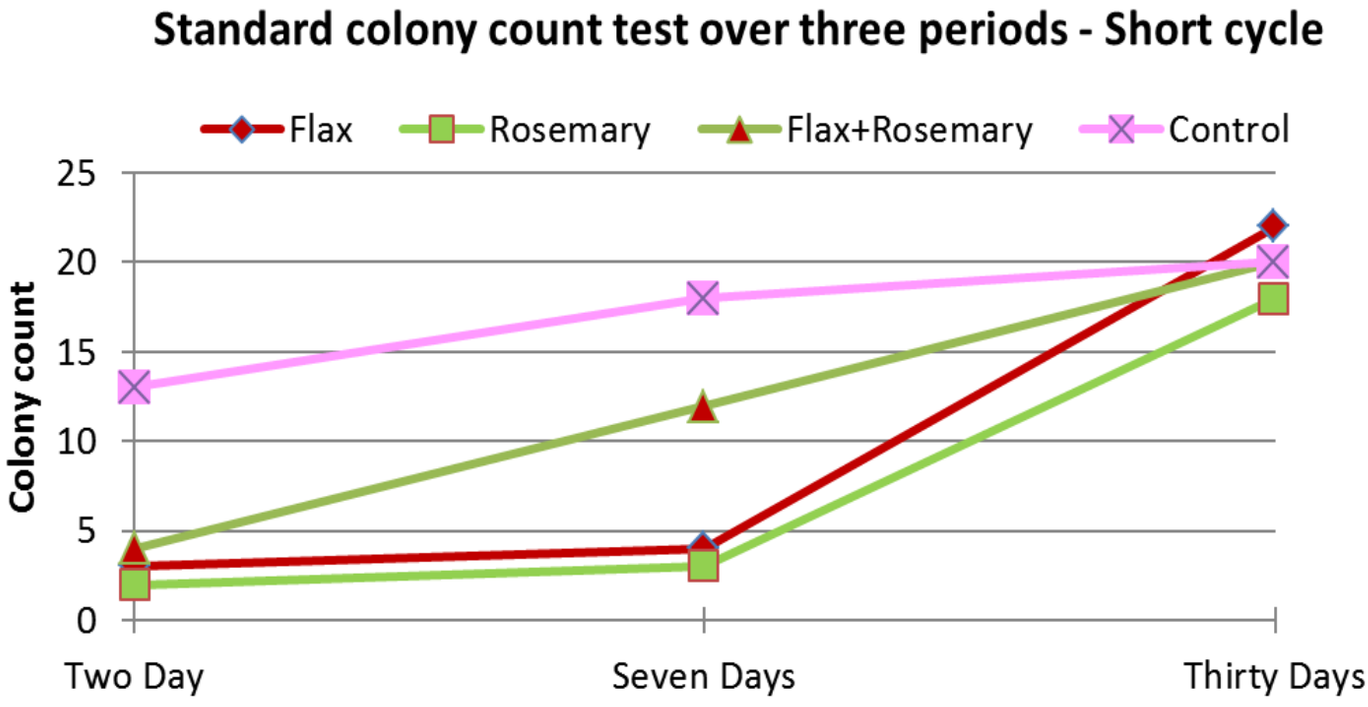

Figure (6). Standard colony count test trend line over three periods of immersion (two, seven, and thirty days) for short cycle.

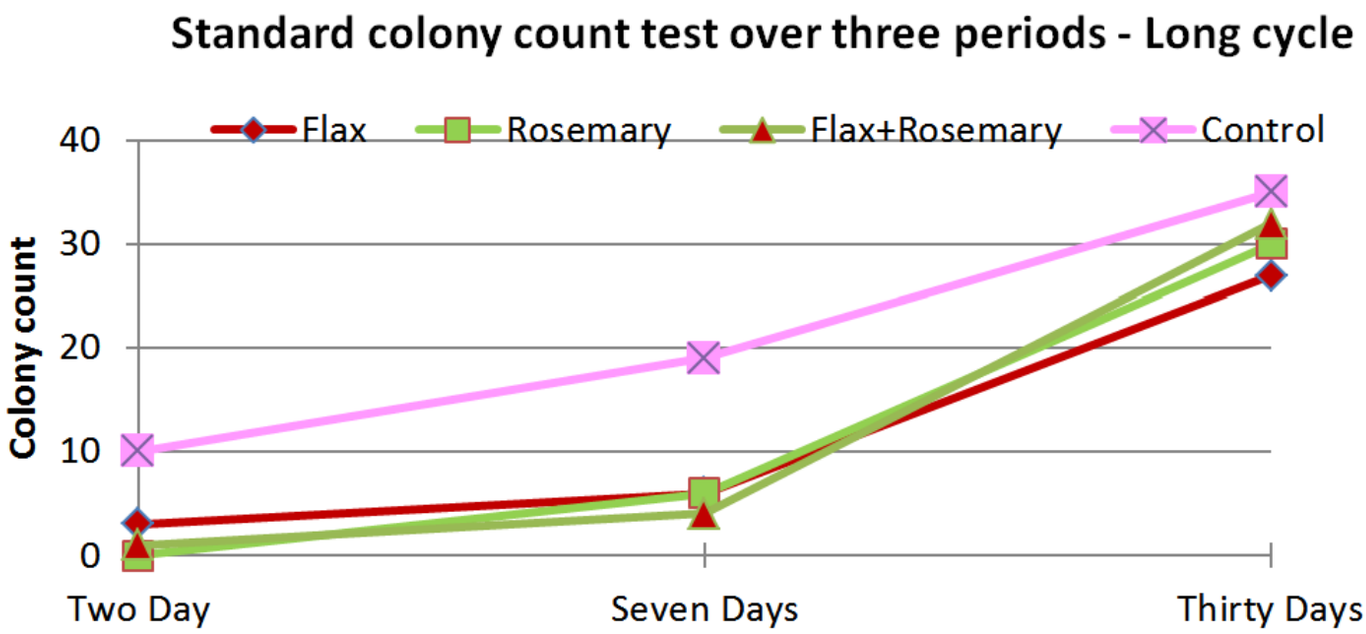

Figure (7). Standard colony count test trend line over three periods of immersion (two, seven, and thirty days) for long cycle.

Figure (8). Standard colony count test for short cycle at two days period.

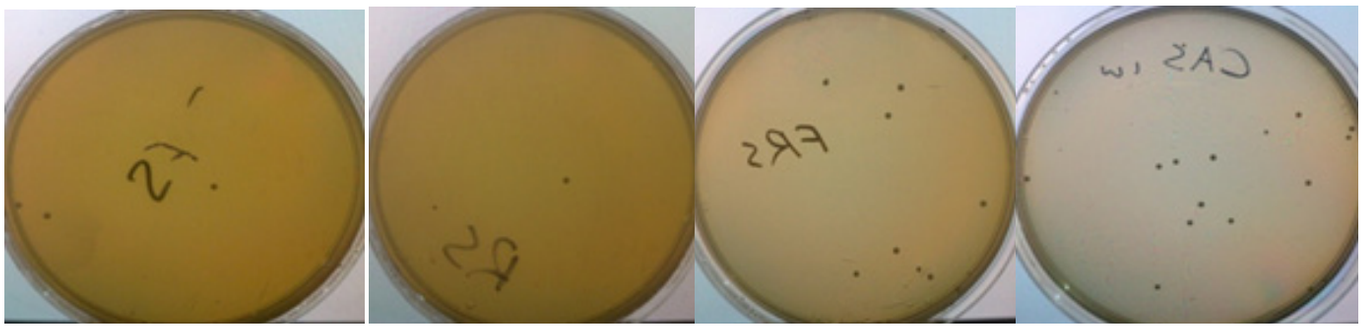

Figure (9). Standard colony count test for short cycle at seven days period.

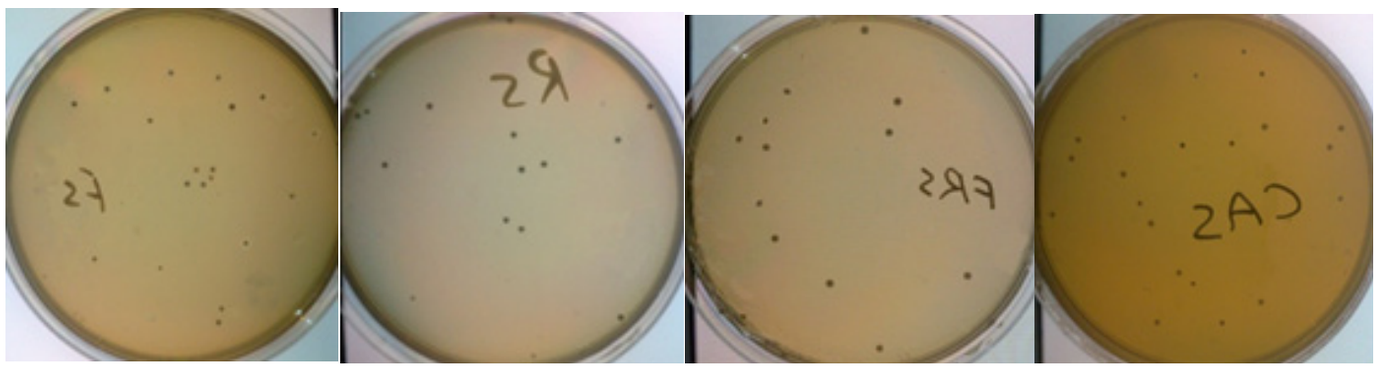

Figure (10). Standard colony count test for short cycle at thirty days period.

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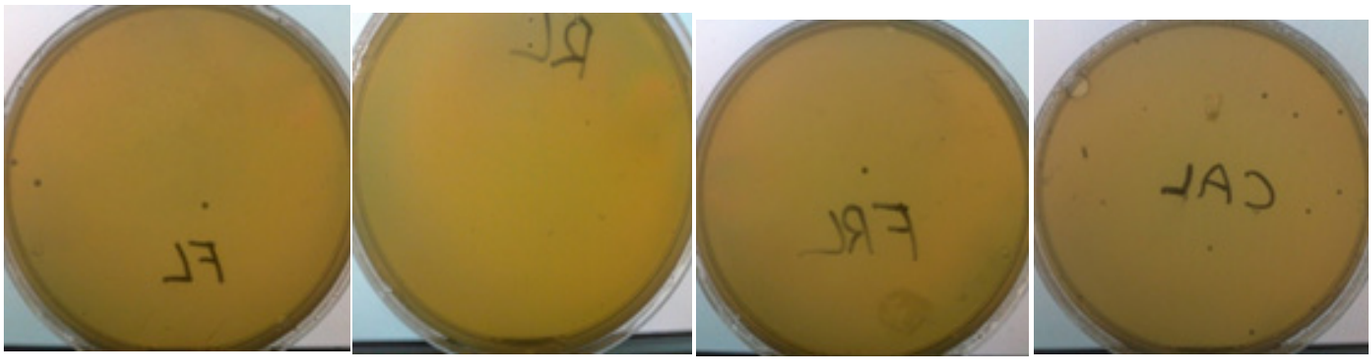

Figure (11). Standard colony count test for long cycle at two days period.

Figure (12). Standard colony count test for long cycle at seven days period.

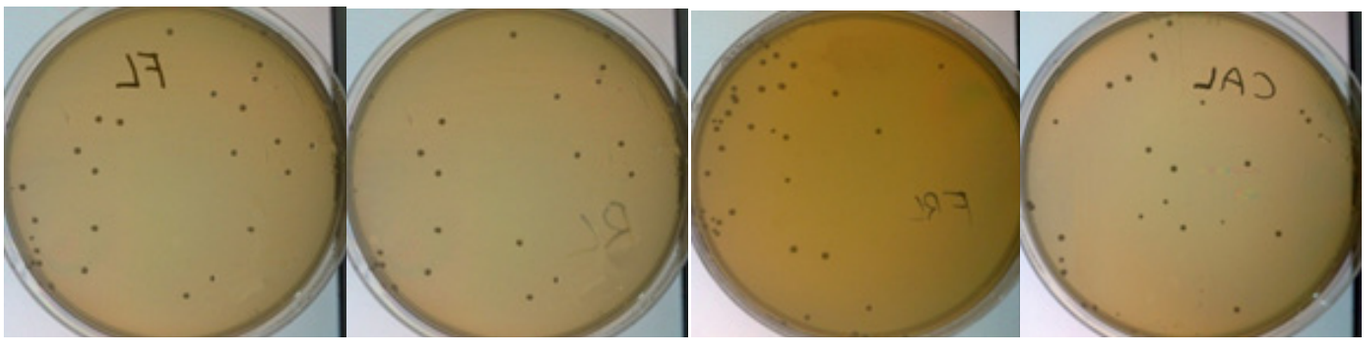

Figure (13). Standard colony count test for long cycle at thirty days period.

Discussion:

Color Stability of Soft Lining Material:

The levels of color change (ΔE) have been evaluated for short cycle groups stored at distilled water for two and thirty days by comparing control group against other three groups as in table (1) and the result was acceptable in vitro and for long cycle groups as in table (2) also the result was acceptable in vitro.

In order to check the effect of immersion time for both short and long cycle caparison was done between two and thirty day, result for short and long cycle groups was acceptable in vivo but not in vitro.

This result can be explained by the chemical structure of soft denture liners. Since, vertex is a polymethyl/ethyl methacrylate with a plasticizer, dibutyl phthalate. Polymethyl/ethyl methacrylate polymer is hydrophilic, attracting water soluble dyes to the surface of the lining material as a result of electrostatic charges [5]. It was stated that plasticized acrylic resin soft liners had high solubility and sorption [26]. In the acrylic resin material, this occurs due to the higher plasticizer solubilisation and consequently higher water absorption [27]. [28] said that the presence of plasticizer in the liner composition increases chain stretching of small organic molecules in the polymer, making diffusion of staining solutions easier. Staining agents may also penetrate the spaces created by leaching of the plasticizer.

Antifungal Activity:

The antifungal activity result inhibition zone of Flax oil, Rosemary oil, nystatin and fluconazole short and Duncan's multiple range tests of it present in figure (5), with fluconazole had the greater inhibition zone followed by nystatin then by rosemary and flax respectively.

The standard colony count result for short and long cycle groups at different immersion time (two,

seven and thirty days) present in figure (7 and 8), showed that in short cycle, the rosemary had the best antifungal followed by flax oil group, this at two and seven days and at thirty days flax oil group followed by mixed then by rosemary groups, while for long cycle rosemary group had the best antifungal followed by mixed group, this is at two and seven days, but for thirty days the flax group had best antifungal activity then followed by rosemary.

The antifungal effects of Rosemary essential oil can be attributed to the Monoterpens combination [29].

Flax oil contains Phenolic acids which are among the phytochemicals (plant chemicals) found abundantly in plants. They appear to have antioxidant, anticancer and antimicrobial activities. Flax contains about 8 to 10 gm of total phenolic acids per kilogram (kg) of flax. Flax contains about 35–70 milligrams (mg) of flavonoids/100 g [30]. Rosemary is credited as antimicrobial (diterpenes). Gram-negative bacteria such as Staphylococcus aureus and S. epidermidis have been found to be more susceptible to rosemary oil than other Gram-negative bacteria such as Escherichia coli and Pseudomonas aeruginosa [31]. The Rosemary oil is very effective against drug-resistant mutants of bacteria and fungi and that it has greater efficacy against fungus than bacteria [32].

Conclusions:

The addition of oils to soft liner results in antifungal activity last within one month and acceptable levels of color change (ΔE) in vitro obtained after oils addition to soft liner (vertex).

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