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- *Acknowledgment:* All contributors who do not meet the criteria for authorship should be listed in an acknowledgments section. Examples of those who might be acknowledged include a person who provides purely technical help, writing assistance, or a head of department who is providing general support.
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About IDJ

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AIMS

Iraqi Dental Journal a peer-reviewed, open access journal that is a published original research articles, review articles, and clinical studies in all branches of dentistry scientific journal dedicated to the dissemixillofacial nation of new knowledge and information on all sciences relevant to dentistry and to the oral and maz structures in health and pathological conditions.

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

Audiance

Postgraduates, undergraduates, members of the dental team, hospitals community, academic & general practitioners.

Iraqi Dental Association



Vision and Mission

It is possible for everyone to succeed, but not all successful people are creative enough to maintain their success. The fall from the top is much easier than falling at the beginning of the ascend, but whoever has a mission can rise and fly-by according to that vision and it is no doubt that he will be a happy successful creator. Rarely heard, a vision owner leaves the summit falling after a rise, because the potential of human being

mentally evolves in creativity whenever the end of the project is clear to him. And as such is our vision and mission in the Iraqi dental journal IDJ. Our Vision: Creativity in modern dentistry.

Our Mission: Education, training, development and scientific creativity to dentists inside and outside Iraq in updated dentistry.

We would like to serve all the people, thus we endeavor that our work reaches them all according to our mission of education and training of beginners, the development of learners and creators support. All based on solid scientific foundations of the process of scientific research, and that will be our gift to them from Mesopotamia.

Join us in our journey to the summit!

in this issue (issue 1.vol.35) of IDJ 14 researcher in dentistry. 36 leaders, consultants and active participants in the progress of Dentistry in Iraq, also Joined us 6 of scientific advisers from outside Iraq, all of them share our vision and mission. we are waiting for your suggestions and your opinions which will help us grow.

Rafi M. Al-Jobory Editor in Chief

The Importance of Evidence Based Journals in Our day to day Clinical Practice.

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It gives me a great pleasure to start this guest editorial by congratulating the editor in chief and the whole editorial team of the Iraqi Dental Journal (IDJ). They are to be commended , not only for their hard work in bringing this peer reviewed journal back to life , but also for their great efforts and focus to make this journal an evidence based dental journal which aims to bridge the gap between science and day to day clinical practice.

Dentists in Iraq and all around the globe deserve to get the truth, the whole truth and nothing but the truth. Therefore, the IDJ team has decided to make of this journal an evidence based journal as this is the only accepted way of delivering the whole truth. Anything, but

evidence based reports and articles might deliver half of the truth, which is often misleading and can have a harmful and adverse effect on our daily clinical practice.

Many opponents of Evidence Based Practice (EBP) believe it to be "cookbook" medicine. However, EBP is not about mindlessly applying the results of research to all clinical settings and all patients. Instead, it promotes the integration of best available evidence with the individual clinician's experience and clinical judgement. In addition to this, the patient's expectations and preferences need to be taken into the decision making process; a factor often not considered. Only when research evidence, clinical expertise and patient values are considered together, can practice truly be classed as evidence-based.

One misconception of EBP is that it focuses entirely on randomised controlled trials (RCTs), which are the gold standard of primary research. RCTs do have an important role to play with EBP, but only when the question to be answered focuses on the effectiveness of a health care intervention. RCTs are not able to answer all research questions and it is important that clinicians are able to recognise the value of different types of primary research for answering different types of clinical questions.

Cohort studies also play a major role in EBP. They measure the incidence of a disease, look at the causes of such disease and determine prognosis. One example of Cohort studies is the study conducted by Esposito et al to investigate if there is any significant difference when comparing the prognosis of dental implants placed in patients with severe periodontitis to those placed in patients with moderate periodontitis and in healthy patients.

Another type of studies used in EBP is the Case- control studies which involve identifying two groups; those who have the outcome of interest (cases) and those who have not (controls). The investigator then looks back in time to see who had the exposure/intervention of interest. One examples of Case control studies is the one conducted by Weng et al to record the clinical outcome for a series of maxillary over-dentures retained by two implant-secured crowns and these outcomes with a control group of over-dentures supported by crowns on two natural canine teeth.

Other important study designs include controlled clinical trials, cross-sectional surveys, correlation studies and case series reports.

Irrespective of the type of research considered to be appropriate for addressing a given clinical question, the research needs to be carefully appraised for validity and applicability before results can be applied in practice.

EBP has had a mixed reception since its 'official' introduction in the early 1990s, with clinicians often reporting that it is either unnecessary or unfeasible in every day practice. Despite the promotion of EBP over the last 20 years, it has been estimated that only 15% of all clinical practice is based on sound research evidence (Bahrami et al, 2004). It has been suggested that the most realistic use of EBP by clinicians involves the use of summaries of research evidence.

This issue of the Iraqi Dental Journal contains six very good articles on various topics in general dentistry and I fully trust you will enjoy reading this issue. Finally, I would like to take this opportunity to invite you all to contribute to your national dental journal and do your part in adding to the development of dentistry in Iraq which will only reflect good on the iraqi community ; patients, dentists, dental students and universities.



Juest Editorial

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Smile Dental Journal has been selected to be one of the sources of input of the Index Medicus for the WHO Eastern Mediterranean Region (IMEMR), Ulrich's, DOAJ, Open J-Gate, Index Copernicus, Portal LivRe & Electronic Journals Library.

Literature review, original research, clinical case reports, case series, short communication, randomized clinical trials, and book reviews are among our scope of published material, where the clinical aspect of dentistry is presented in a scientific way, starting each article with an abstract, backed up by references in accordance with the Vancouver citation style.

One of our major concerns is the review process of the papers prior to their publication. Peer-review is a process of revision that insures accuracy, clarity, and smooth readability of these papers. In Smile's editorial policy we adopt the double blinded peer review, where both the reviewer and author are kept anonymous. Manuscripts are reviewed on a double-blind basis by two reviewers from the editorial review board of Smile and/or by external reviewers depending on the manuscript content and specialty.

We eagerly look forward to the cooperation between Smile Dental Journal and The Iraqi Dental Association, that will surely enrich Smile's scientific content with high quality, well structured dental articles from our Iraqi colleagues that will be a great benefit to our readers all over the world, from one side, and on the other side will help our Iraqi colleagues to get the maximum exposure for their published articles that they deserve.

Best regards,

Dr. Issa Bader Founder & Editor-in-Charge Smile Dental Journal



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Effect of Thyme Water Extract on Commonly Found Oral and Root Canal Bacteria (A Comparative Study)

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ABSTRACT

Background and objectives: Bacteria and their products play an essential role in the pathogenesis of pulpo-periapical disease. The purpose of this study was to compare the anti-microbial activity of thyme water extract on selected bacterial isolates and cultivated root canal swabs in comparison with different concentrations of sodium hypochlorite (NaOCI).

Materials and Methods: Clinical isolates of *Staphylococcus aureus, Moraxella catarrhalis,* and *Klebsiella pneumoniae* were selected. Microbiological samples from infected root canals were taken from forty five male and female patients in age group of 15-45 years old during the first visit of root canal therapy. Antibacterial assay were done by agar well diffusion method and microdilution assay.

Results: The best antimicrobial activity for thyme extract was noticed at *Moraxella catarrhalis* and failed to show any significant effect on *Klebsiella pneumoniae*. The results revealed that 2% sodium hypochlorite are more effective than thyme water extract, on evaluated bacterial isolates and swabs.

Conclusion: From bacteriological point of view, it could be demonstrated that water extracts of thyme (infusion method), may be useful for root canal irrigation with their antibacterial effect.

KEYWORDS: Thyme, Irrigant, Sodium hypochlorite, Root canal bacteria.

INTRODUCTION:

The goal, with the great majority of teeth required root canal treatment, is either prevention or treatment of apical periodontitis. In other words, the goal is prevention and elimination of a microbial infection in the root canal system.⁽¹⁾ Bacteria and their products play an essential role in the pathogenesis of pulpo-periapical disease.⁽²⁾ Eradication of microorganisms from an infected root canal system has been demonstrated in numerous studies to be the key to successful endodontic treatment.^(3,4)

Although root canal irrigants such as sodium hypochlorite appear to be effective at reducing bacterial cultures, most of the previous studies failed to adequately report these clinically important and potentially patient-relevant outcomes. There is currently insufficient reliable evidence showing the superiority of any one individual irrigant.⁽⁵⁾

The pulpo-dentin complex is normally protected from the oral cavity by the overlying enamel or cementum. Once caries, trauma, or restorative or periodontal procedures breach the integrity of this barrier, the tubules provide diffusion channels from the surface to the pulp. Bacteria can then invade these dentinal tubules, and bacterial products can diffuse across dentin to elicit pulpal reactions.⁽⁶⁾ All bacteria within the oral cavity share the same opportunities for invading the root canal space, however only a restricted group of species have been identified in infected root canals.⁽⁷⁾ The predominant microbial groups frequently isolated from infected root canal are the aerobic and facultative anaerobic organisms.^(8,9) These types of organisms frequently isolated more than obligate anaerobic species in developed periapical lesion, during the standard culture technique.^(9,10)

Aromatic plants are promising sources of natural antimicrobial and antioxidant compounds. These properties may be associated with their bioactivities and health effects.⁽¹¹⁾ Thyme (*Thymus vulgaris L.*) is an aromatic plant known by its antioxidant and antimicrobial activity, both related to the phenolic compounds, particularly *thymol* and *p-cymene*, the most abundant compounds in thyme leaves.^(12,13) It is used orally to treat dyspepsia and other gastrointestinal disturbances; coughs due to colds, bronchitis and pertussis; and laryngitis and tonsillitis (as a gargle). Topical applications of thyme extract have been used in the treatment of minor wounds, the common cold, disorders of the oral cavity, and as an antibacterial agent in oral hygiene.^(14,15)

The purpose of this study was to evaluate *in vitro*, the antimicrobial activity of thyme water extract, in comparison with sodium hypochlorite

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against selected bacterial isolates and against swabs taken clinically from prepared root canals in patients presenting for endodontic treatment.

MATERIALS & METHODS

Determination of Plant Material

Five hundred grams of fresh plant material were purchased from the local botanical market of Sulaimani and were identified by the Herbarium of the College of Agricultural, Sulaimani University.

Preparation of the Plant Extract

Extraction of fresh plant material was performed by hot water extraction, without drying the plant parts according to Goyal, *et al.* (2008).⁽¹⁶⁾ The infusion prepared by boiling 10 g of plant parts (leaf, stem and root) in distilled water with constant stirring for 30min. The solution was allowed to cool to room temperature and then filtered using muslin cloth. The filtrate was centrifuged at 500rpm for 15min. The supernatant was again filtered using Whatman filter paper (No.1) under strict aseptic condition. The filtrate was collected in fresh sterilized glass tubes and stored at 40C until use.

Test Organisms

Clinical isolates of *Staphylococcus aureus, Moraxella catarrhalis*, and *Klebsiella pneumoniae* were used in this study. The microorganisms were provided by microbiology laboratory of the Biology Department, College of Science, Sulaimani University.

Root Canal Sampling

Forty-five male and female patients in age group of 15-45 years attending dental clinic at Conservative Department, College of Dentistry, University of Sulaimani, were taken for this study. Each patient included in study was having at least one intact or necrotic open canal tooth. As a diagnostic measure, for each of the suspected tooth an intra oral periapical X-ray was taken.

Forty-five endodontic samplings from infected root canal were obtained during the first visit of root canal therapy by a sterile file. Sampling was also performed to check and confirm the sterility of the operating field before intracanal sampling procedure, and then transferred to sterile 2ml tubes containing VMGA III transport medium. All samples were processed within 2 hours, and cultivated over a suitable growth media. The culture media used were nutrient agar, Mueller Hinton agar, MacConkey agar, Cetrimide agar, Simmon citrate agar and peptone water (Biotech Laboratories United Kingdom), Thioglycolate broth (Maknur, Canada), Trypticase Soy broth (Becton Dickinson, USA), and the media were prepared according to the manufacturers' specifications. Two sets of plates with different growth media were equally prepared for each patient, one for aerobic incubation, and the other for anaerobic incubation.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations (MIC) of thyme water extract and NaOCl, against the test bacteria were determined by standard two-fold microdilution methodology as described by Levinson (2004).⁽¹⁷⁾ Thioglycolate medium fluid was prepared according to the manufacturer's instructions. Different concentration of thyme water extract were made from crude extracts (200, 250, 500, 750, 1000, 1500) mg/ml. The dilution procedure was carried out using micropipettes. A sterilized screw-capped tube was used for the dilution, the total volume of contents of each tube after dilution was 4ml.

Antibacterial Activity

An in vitro agar well diffusion assay was performed, to test the susceptibility of selected clinical isolates and sub-cultured root canal swabs to thyme water extract and NaOCl.⁽¹⁸⁾ Fifteen milliliters of the molten agar (45 °C) were poured into sterile petri dishes (Ø 90mm).50 µl from each selected bacterial isolate and from the swabs (Cell suspensions containing 10⁸ CFU/ml cells), were taken separately and evenly spread onto the surface of the agar plates of Mueller-Hinton agar (Oxoid, UK), using a micropipette. Five plates for each microorganism were inoculated. For each swab two sets of plates with different growth media were inoculated, one for aerobic incubation, and the other for anaerobic incubation. Once the plates had been aseptically dried, 6mm wells were bored using a sterile cork borer. Equal amount (75 µl) of thyme water extract (1500mg/ml), 1%NaOCl and 2%NaOCl were placed into the wells and the plates were incubated at 37°C for 24h.

Statistical Analysis

Growth inhibition zones were calculated. Student *t*-test was applied to determine significant differences between the test and control solutions. Significance was predetermined at P<0.05. Statistical analysis was performed with the SPSS statistical software package.

RESULTS

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The descriptive statistics (mean and standard deviation) of the inhibition zones (in millimeter) around the wells for thyme water extract (1500mg/ ml), 1%NaOCl and 2%NaOCl against the selected bacterial isolates, and against swabs taken from prepared root canals that were incubated aerobically and anaerobically, are shown in table 1. The best antimicrobial activity of thyme extract and both concentrations of NaOCl were noticed at *Moraxella catarrhalis*. The results revealed that 2% NaOCl had more antimicrobial activity (p<0.05) than thyme and 1% NaOCl on the tested microorganisms and the swabs. Also, it was observed that thyme extract was failed to show any significant effect on *Klebsiella pneumonia*.

It significantly (p < 0.05) exhibited a wider inhibition zones than 1% NaOCl against *Staphylococcus aureus* and swabs (anaerobic), but it did so to a lesser extent against *Moraxella catarrhalis* and swabs (aerobic).

Concerning the different concentration of thyme water extract, the limits of minimal inhibitory concentration are between 650-1500mg/ml. The 0.1% NaOCl was determined as a minimum concentration required for inhibiting *Staphylococcus aureus* and *Moraxella catarrhalis*. The MIC of NaOCl for *Klebsiella pneumoniae* and swabs was 1%.

(Table 1) Descriptive statistics (mean and standard deviation) of the inhibition zones (in millimeter) of the thyme water extract, 1% NaOCl and 2%NaOCl against bacterial isolates and swabs.

Test organisms	Thyme(1500 mg/ml) Mean±SD	1% NaOCl Mean±SD	2% NaOCl Mean±SD
Moraxella catarrhalis Neisseria catarrhalis	14 ± 1	12 ±0.71	15.6±1.14
Klebsiella pneumoniae	NE	7 ± 0.71	8.4 ± 0.89
Staphylococcus aureus	9 ± 0.71	7.8 ± 1.3	12.2 ± 0.84
Swab(aerobic)	7.71 ±0.73	7.69 ± 0.7	13.64 ±1.33
Swab(anaerobic)	12.42 ±1.44	8.13 ± 1.06	13.58 ±1.45

NE: the test solution was not effective.

DISCUSSION

The use of antimicrobial agent is still considered the fundamental principles during endodontic treatment. None of the chemomechanical methods of root canal treatment is at present able to eliminate all the bacteria from inside the tooth.⁽¹⁾ Available literature and studies demonstrated advantages and limitations of each irrigant under consideration, and none of them satisfy the requirements of the ideal root canal irrigant completely. The search for an ideal root canal irrigant continues with the development of newer materials and methods.⁽¹⁹⁾

Therein lies, the overall purpose of this study was a trail to find a natural antimicrobial agent to be used as an adjunct during chemomechanical root canal preparation, to eliminate the uses of chemical irrigants with their cytotoxic effects. It was planned to conduct a microbiological study, with the aim of determining the minimum inhibitory concentration (MIC), and to assess the antibacterial effect of our test solution (thyme water extract), and to compare it with that of sodium hypochlorite (as a control), whose antibacterial action is well-established in the literature. Sodium hypochlorite, at different concentrations, has been used in endodontic due to its tissue dissolution ability and antimicrobial action.⁽²⁰⁾ The results of the present study supported the antimicrobial efficacy of sodium hypochlorite on the evaluated microbiota.

All clinical isolates were selected for the present study to represent different groups of organisms, including Gram-positive facultative anaerobic (Staphylococcus aureus), Gram-negative facultative anaerobic (Klebsiella pneumoniae) and Gram-negative aerobes (Moraxella catarrhalis). The variation in the antimicrobial action of thyme and NaOCl against different microorganisms could be attributed to the differences in the mode of metabolism of each bacterial isolate, and the differences in the mode of action of each agent, that's some microorganisms, are with respiratory metabolism, some with fermentative metabolism, and some with both. The zones of inhibition may be affected by the diffusibility of the agent through the agar media. Therefore, the size of the inhibition zones does not indicate the absolute antimicrobial effect of a solution.

Our results showed that there were statistically significant differences in the antimicrobial action between the thyme water extract and 2%NaOCl. The data obtained, clearly showed that their antibacterial properties are lower than sodium hypochlorite, for that reason it cannot replace sodium hypochlorite.

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This may be due to the fact that our extraction method was suboptimal for obtaining high concentrations of active agents in the extracts. It is therefore, recommended that future investigation will incorporate sequential distillation of mixtures of herbs from different countries, to ensure adequate cover for environmental factors which may affect the level of active ingredients in the extracts. Thymol and its isomer carvacrol, components derived from thyme are classified as monoterpene phenols and have already proven their antimicrobial effect.⁽²¹⁾ Other experimental evidence suggests that the *in vitro* activities of thyme preparations are due to the presence of polymethoxy flavones that have antibacterial activity.⁽²²⁾

In any case, antimicrobial activity is not the only requirement for an endodontic irrigant. Irrigating solutions also possessing the ability to dissolve organic material are desirable in endodontic treatment. When analyzing the antibacterial action of an endodontic irrigating solution, it is necessary to consider its ability to wet the dentin and penetrate dentinal tubules and also its capacity to disrupt the biofilm community.⁽²³⁾ Therefore, there is need for further studies and modifications in thyme water extract (infusion method), to evaluate their biological behaviors on periapical tissues, before it could be used as a root canal irrigant.

CONCLUSION

The present in-vitro study results showed that water extracts of thyme (infusion method), possess antibacterial activities, it might come in useful during chemo-mechanical root canal preparation.

CONFLICT OF INTERESTS

The authors deny any conflict of interests.

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In Vitro Evaluation of the Effect of Thermocycling on the Shear Bond Strength of Light-Cured Composite and Resin Modified Glass Ionomer Cement Using Different Orthodontic Brackets

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ABSTRACT

Background: This study was conducted to investigate and compare the effects of thermocycling on the shear bond strength of two light cured orthodontic bonding materials, namely composite and resin-modified glass ionomer cement that used to bond stainless steel and Sapphire brackets on human teeth, and to determine the type of bonding failure of these materials.

Materials and Methods: Eighty extracted human premolars were selected and randomly divided into two equal groups each with 40 teeth according to the brackets types. Both groups were subdivided into four groups (n = 10) two of these subgroups bonded with composite one control and the second thermocycled and the other two subgroups bonded with resin-modified glass ionomer cement also one control and the second thermocycled. The sample were stored in water at 37° C for 24 hours, then the control groups were tested while the study groups were thermocycled between 5° C and 55° C for 500 cycles. The samples were tested for shear bond strength using an Instron universal testing machine. For adhesive remnant index, the enamel surface and bracket base of each tooth were inspected under magnifying lens (20X) of a stereomicroscope.

Results and Conclusions: The shear bond strength of light-cured composite is higher significantly than resin-modified glass ionomer cement. Thermocycling decreases the shear bond strength of the sapphire brackets bonded with composite significantly by 60.16%. The most predominant sites of bond failure are within the adhesive itself and between the adhesive and the enamel.

KEYWORDS: Shear bond strength, Thermocycling, Resin-modified glass ionomer cement, Composite.

INTRODUCTION

After the introduction of acid etch technique and using composite as orthodontic adhesive, the problems of teeth decalcification, gingival inflammation, caries and many problems associated with banded fixed orthodontic appliances were reduced but decalcification around the orthodontic brackets remains the major problem until the introduction of glass ionomer cement (GIC) which has less harmful effect of dental enamel, release fluoride and has good adhesive properties. Also, GIC has the ability to absorb fluoride from topical fluoride applications. This feature allows it to act as a long-term fluoride releasing agent.

Antonucci *et al.*⁽¹⁾ introduced resin-modified glass ionomer cements (RMGICs) which set through a combination of acid–base reaction and photochemical polymerization.

"Resin-modified" refers to all cements in which the acid–base reaction of true glass-ionomer cements is supplemented by a polymerization reaction.⁽²⁾

In their simplest form, these are glass ionomer cements (GICs) with the addition of a small quantity of a resin such as hydroxyethyl methacrylate (HEMA) or Bis – GMA in the liquid as a co-solvent to avoid phase separation of the resin from the glass-ionomer components.^(3,4)

The fundamental acid/base curing reaction is supplemented by a second curing process, which is initiated by light or chemical. These products are considered to be dual – cure cements if only one polymerization mechanism is used; if both mechanisms are used, they are considered to be tri – cure cements. These new materials are called as resin modified glass ionomer cements or hybrid ionomers. These materials are defined as hybrid materials that retain a significant acid – base reaction as part of their overall curing process.⁽⁵⁾ Actually, resin modified GIC lies between pure resins composite and pure GIC.⁽⁶⁾

Arici and Arici⁽⁷⁾ investigated the effects of thermocycling between 5°C and 55°C for 200 and 20,000 cycles, before testing, on the shear bond strength of chemically cured resin-modified glass ionomer cement in comparison with no-mix composite used to bond metal brackets. They found that there were very high significant differences between the mean shear bond strengths of the groups. For the resin-modified glass ionomer cement groups, the predominant bond failure site was at the bracket-adhesive interface.

In Iraq, four studies⁽⁸⁻¹¹⁾ evaluated the shear bond strength of resin-modified glass ionomer ce-

ment but no study evaluated the effect of thermocycling on the shear bond strength except for one study that evaluate the thermocycling on the shear bond strength of two types of self- etch primers;⁽¹²⁾ so this *in vitro* study was carried out to investigate the effects of thermocycling on the shear bond strengths of a light cured resin-modified glass ionomer cement used for bonding of orthodontic brackets and to compare this bonding agent with a light cured conventional composite resin. The bond failure sites were also investigated.

MATERIALS & METHODS

Teeth

Eighty freshly extracted human premolars were collected and stored in a solution of 0.1% (weight/volume) thymol. The criteria for tooth selection included intact buccal enamel that had not been subjected to any pretreatment chemical agents, e.g. hydrogen peroxide, with no cracks due to the pressure of the extraction forceps, and no caries.

Retentive wedge shaped cuts were made along the sides of the roots of each tooth to increase the retention of the teeth inside the self-cured acrylic blocks. Each tooth was fixed on a glass slide in a vertical position using soft sticky wax at the root apex, so that the middle third of the buccal surface was oriented to be parallel to the analyzing rod of the surveyor. This kept the buccal surface of tooth parallel to the applied force during the shear test.⁽¹³⁾ Then the two L-shaped metal plates, were painted with a thin layer of separating medium (Vaseline) and placed opposite to each other in such way to form a box around the vertically positioned tooth with the crowns protruding. After that, the powder and liquid of the self cured acrylic were mixed and poured around the tooth to the level of the cemento-enamel junction of each tooth.⁽¹⁴⁾ After setting of the self cured acrylic resin, the two L-shaped metal plates were removed, the sticky wax used for fixation of tooth in the proper orientation removed too and the resulting holes filled with self cure acrylic. Slight adjustment of the acrylic blocks was done using the portable engine to adjust the acrylic block to make it fit properly in the testing machine. After mounting, the specimens were color coded and stored in normal saline solution with thymol to prevent dehydration until bonding.⁽¹⁵⁾

Brackets

Two types of 0.022" MBT orthodontic brackets were used in this study: Stainless-steel brackets {Mini-sprint[®]} from Forestadent Co., Germany and Sapphire brackets {Perfect SB (clear[®])} from Hubit Co., South Korea with base surface area 8.92mm² and 12.807mm² respectively.

The selected eighty teeth were randomly divided into two main groups 40 teeth of each on the basis of type of brackets: Group A: Stainless-steel brackets {Mini-sprint[®]} and Group B Sapphire brackets {Perfect SB (clear[®])}. Both groups were subdivided into four groups (n = 10) according whether thermocycled or not:

- Group (AI): stainless steel brackets bonded with light cured composite and stored in water at 37°C for 24 hours.
- Group (AII): stainless steel brackets bonded with light cured composite and thermocycled for 500 cycles.
- Group (AIII): stainless steel brackets bonded with light cured RMGIC and stored in water at 37°C for 24 hours.
- Group (AIV): stainless steel brackets bonded with light cured RMGIC and thermocycled for 500 cycles.
- Group (BI): Sapphire brackets bonded with light cured composite and stored in water at 37°C for 24 hours.
- Group (BII): Sapphire brackets bonded with light cured composite and thermocycled for 500 cycles.
- Group (BIII): Sapphire brackets bonded with light cured RMGIC and stored in water at 37°C for 24 hours.
- Group (BIV): Sapphire brackets bonded with light cured RMGIC and thermocycled for 500 cycles.

Bonding and Thermocycling

The teeth were cleansed and then polished with pumice slurry and rubber prophylactic cups for 10 seconds then thoroughly washed and dried.⁽¹⁶⁾

For the composite, (according to the manufacturer's instructions) 37% phosphoric acid gel was applied for 30 seconds, washed with air water spray for 20 seconds and then dried with oil/ moisture-free air until the buccal surface of the etched tooth appeared chalky white in color. Thin uniform coat of Resilience[®] sealant (Ortho technology Co., USA) were applied by brush on each tooth surface to be bonded. Small increment of Resilience[®] adhesive paste (Ortho technology Co., USA) then applied onto the bracket back using flat ended instrument.

For the RMGIC (GC Fuji Ortho LC, GC Corporation/Japan), also according to the manufacturer's instructions, the standard powder to liquid ratio was 3.0g/1.0g was mixed (1 level large scoop of powder to 2 drops of liquid) which was mixed by dividing the powder into two equal parts; the first part was mixed with all the liquid and mix for about 10 seconds. Then the other part of powder was incorporated and mixed

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thoroughly for an additional 10-15 seconds (total mixing is 25-30 seconds) the final mixture was having creamy honey-like consistency. Immediately after applying the adhesive to the bracket base, the bracket was placed gently onto the centre of the labial surface using a clamping tweezers.

A load of about 300g was attached to the vertical arm of the surveyor to standardize the pressure applied on the brackets during bonding to ensure seating under an equal force and to ensure a uniform thickness of the adhesive and prevent air entrapment which may affect bond strength.⁽¹⁷⁾ The excess then removed from around the bracket with dental probe.

Flash Max 2 light cure unit (CSM dental Aps, Denmark) uses a 15 Watt diode. This super LED has an optical out-put well above 4.000 mW/cm² was used to cure the two types of adhesives. Six seconds; three seconds from mesial and three seconds from distal sides used to cure the adhesives with a minimum separation distance (1-2) mm. Every tooth was left undisturbed for 30 minutes to ensure complete polymerization of adhesive material.⁽¹⁸⁾

After bonding, all samples stored in water at 37°C for 24 hours. The control group tested after that while the study samples were thermocycled between 5°C and 55°C for 500 complete cycles. The thermocycling was done manually following the recommendation of the international organization for standardization (ISO/TS 11405), the exposure to each bath was 30 seconds, and the transfer time between the two baths was 5-10 seconds.⁽¹⁹⁾

De-bonding & Examination of Adhesives Remnants

The samples were tested for shear bond strength using an Instron universal testing machine. A crosshead speed of 0.5mm/minute was used. Readings were recorded in Newtons. The force was divided by the surface area of the bracket base to obtain the stress value in Mega Pascal units.

To estimate the adhesive remnant index, the debonded brackets and the enamel surface of each tooth were inspected under a stereomicroscope (magnification 20X) to determine the predominant site of bond failure. The site of bond failure was scored according to Wang *et al.* classification⁽²⁰⁾ and as followed:

• Score I: The site of bond failure was between the bracket base and the adhesive.

• Score II: Cohesive failure within the adhesive itself, with some of the adhesive remained on the tooth surface and some remained on the bracket base.

• Score III: The site of bond failure was between the

adhesive and the enamel.

• Score IV: Enamel detachment.

Statistical Analysis

Data were collected and analyzed using SPSS software version 15 (2006). In this study the following statistics were used:

a) Descriptive statistics: including means, standard deviations and percentages.

b) Inferential statistics: including independent sample *t*-test: to test any statistically significant difference of the shear bond strengths between groups.

In the statistical evaluation, the following levels of significance are used:

Non-significant	NS	<i>P</i> > 0.05
Significant	*	$0.05 \ge P > 0.01$
Highly significant	**	$0.01 \ge P > 0.001$
Very highly significant	***	$P \le 0.001$

RESULTS & DISCUSSION

A balance in bond strength must be achieved when choosing a bracket-adhesive combination for fixed orthodontic treatment. Bond strength should not only be high enough to resist the forces during the course of orthodontic treatment but also low enough to allow the removal of the bracket without any complications at the end of orthodontic treatment. Therefore, high mean bond strength does not necessarily mean better clinical performance.⁽⁷⁾

The findings of this study can not be thoroughly compared with other studies due to different thermocycling protocol, different adhesives, new light cure unit used with less curing time and sapphire brackets are used for the first time in thermocycling researches.

The results of this study indicated that the SBS of sapphire brackets bonded with light-cured composite and RMGIC is higher than stainless steel brackets in both control and thermocycling groups with a very high significant difference (Table 1). This could be explained by the translucency of sapphire brackets that gives a better chance for a more complete polymerization with light curing in addition to the presence of zirconia particles coating the bracket base that creates millions of undercuts that secure the bracket in place due to the micro-mechanical retention means.

In both types of brackets and adhesives, the SBS in thermocycling group is lower than control group with a non-significant difference except for

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sapphire brackets bonded with light-cured composite where there is very high significant difference between control and thermocycling groups (Table 1). This may possibly be explained by the absorption of water and the alternating stressing of the system resulting from the large mismatch of the thermal expansion coefficients of the adhesives, brackets and enamel. These differences between the thermal coefficients of the three components of the system are likely to affect adversely the adhesion of the resin to other parts of the system. The cyclical stress may cause any de-bonded regions at the interfaces to grow progressively in size. Because the RMGIC consists of a mixture of two components, namely, glass ionomer and resin adhesive, this extra interface between the two might make this cement more prone to this adverse effect; this comes in agreement with the findings of Arici and Arici.⁽⁷⁾

As shown in table 1, the percentages of reduction of SBS after thermocycling in stainless steel and sapphire brackets bonded with light-cured composite are 3.52% and 60.16% respectively. On the other hand, the same brackets bonded with light-cured RMGIC showed approximately the same percentages (4.28% and 4.36% respectively).

Comparing the SBS of stainless steel and sapphire brackets, separately, bonded with both types of adhesives reveal that it is higher in composite than RMGIC in control and thermocycling groups with a very high significant difference (Table 2). This indicates that composite is better than RMGIC in this study although some studies agree and others disagree with this result. Increased in the SBS of composite may be attributed to the effect of acid etching of enamel that significantly increased the bond strength of brackets to enamel.

Generally, the mean value of SBS of stainless steel bonded with light-cured composite in this study is nearly similar to that of Garma⁽²¹⁾ but for sapphire brackets, it is higher. This may be attributed to the difference in the surface areas of the brackets although the same adhesive, light cure unit and curing time are used.

The site of failure provides useful information about the bonding process. Ideally, in orthodontics, an adequate bond that fails at the enamel-cement interface is desirable because de-bonding and subsequent polishing procedures would become much easier.⁽²²⁾

Reviewing tables 3 and 4 reveal that in sapphire and stainless steel brackets bonded with RMGIC, the predominant score is III that is most of the adhesive remained on the brackets because RMGIC bonds better to the base of the bracket than to enamel; this comes in agreement with the findings of Toledano *et al.*⁽²²⁾ while disagrees with Arici and Arici.⁽⁷⁾

For sapphire brackets bonded with composite, the predominant scores are scores II and III while for stainless steel brackets the predominant score is score II with 20% score I. With the use of acid-etching technique, almost none of the bonding failures were located at the resin-enamel interface. This may be attributed to the incomplete polymerization of the resin just below the metal base of the bracket. In sapphire brackets score I is absent because of their translucency.

The occurrence of these types of failure sites (scores II and III) may offer a clinical advantage in protecting the adhesive enamel interface from damage. On the other hand, this reduces teeth cleaning time and is less bothersome for the patients.

CONCLUSIONS

- 1. The shear bond strength of light-cured composite is higher significantly than resin-modified glass ionomer cement.
- 2. The shear bond strength of sapphire brackets bonded with light-cured composite and RMGIC is higher than stainless steel brackets in both control and thermocycling groups with a very high significant difference.
- 3. Thermocycling decreases the shear bond strength of the sapphire brackets bonded with composite significantly by 60.16%.
- 4. The most predominant sites of bond failure are within the adhesive itself and between the adhesive and the enamel.

(Table 1) Descriptive statistics of shear bond strength and groups differences according to brackets types and thermocycling

Adhesives	Groups	Descriptive statistics			Group	s difference	
		Stainless steel brackets		Sapphire brackets			
		Mean	S.D.	Mean	S.D.	t-test	P-value
Composite	Control	9.19	0.62	33.66	1.69	-33.52	0.000 ***
	Thermocycling	8.89	1.28	13.43	1.35	-5.99	0.000 ***
	t-test	0.:	53	23.	04		
	P-value	0.61	(NS)	0.000) ***		
% of reducti	on after thermocycling	3.52%		60.16%			
RMGIC	Control	3.45	0.25	4.67	0.39	-6.31	0.000 ***
	Thermocycling	3.28	0.16	4.44	0.49	-5.52	0.000 ***
	t-test	1.	39	0.8	87		
	P-value	0.19	(NS)	0.41	(NS)		
% of reducti	on after thermocycling	4.28%		4.3	6%		

(Table 2) Descriptive statistics and groups differences according to adhesives types

Groups	Stainless steel brackets					S	apphire	brack	xets			
	Comp	osite	RMO	GIC	t-test	P-val- ue	Comp	osite	RMO	GIC	t-test	P- value
	Mean	S.D.	Mean	S.D.			Mean	S.D.	Mean	S.D.		
Control	9.19	0.62	3.45	0.25	21.05	0.000 ***	33.66	1.69	4.67	0.39	41.19	0.000 ***
Thermocy- cling	8.89	1.28	3.28	0.16	10.61	0.000 ***	13.43	1.35	4.44	0.49	15.37	0.000 ***

(Table 3) Frequency and percentage of occurrence of the adhesive remnant index (ARI) for stainless steel brackets group

Groups	Composite		RMGIC	
Scores	Control	Thermocycling	Control	Thermocycling
Ι	2(20%)	2 (20%)	0 (0%)	0 (0%)
II	8 (80%)	8 (80%)	0 (0%)	0 (0%)
III	0 (0%)	0 (0%)	10 (100%)	10 (100%)
IV	0 (0%)	0 (0%)	0 (0%)	0 (0%)

(Table 4) Frequency and percentage of occurrence of the adhesive remnant index (ARI) for sapphire brackets group

Groups	Composite		RMGIC	
Scores	Control	Thermocycling	Control	Thermocycling
Ι	0 (0%)	0 (0%)	0 (0%)	0 (0%)
II	5 (50%)	6 (60%)	0 (0%)	0 (0%)
III	5 (50%)	4 (40%)	10 (100%)	10 (100%)
IV	0 (0%)	0 (0%)	0 (0%)	0 (0%)

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The Beneficial Use of Nigella Sativa Powder in the Healing of Different Portions of Extracted Tooth Socket: In Vivo Study

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ABSTRACT

Back ground: Nigella sativa is so beneficial due to it's content of over a hundred components such as aromatic oils, trace elements, vitamins and enzymes. It contains 58% of essential fatty acids including omega 6 and omega 3. These are necessary for the forming of Prostaglandin E1 which balances and strengthens the immune system giving it the power to prevent infections and allergies and control chronic illnesses. Blackseed oil also contains about 0.5 - 1.5% volatile oils including nigellone and thymochinone which are responsible for its anti-histamine, anti-oxidant, anti-infective and broncho-dilating effect.

Aim of the study: To evaluate the effect of Nigella sativa (powder and oil) on the healing process in different portions of rabbit socket by histomorphometric analysis of bone architecture parameters.

Materials and Methods: The sample of the study consist of twenty rabbits, extract their upper two central incisors under general anesthesia. The left side filled once with Nigella sativa powder and once with Nigella sativa oil material, and the right side left for normal healing as a control group. The two sockets were sutured. The results were studied in intervals period 2 and 6 weeks, histologically and then assayed for histomorphometic analysis for trabecular width, trabecular number and the trabecular separation Marrow space star volume analysis (V*m), Osteoblast, osteocyte, and blood vessels number, in coronal, middle and apical portions of extracted tooth socket. These parameters are derived from microscopic two dimension image measurements analyzer software program.

Results: Histomorphometric analysis for variables of trabeculae width (thickness of trabeculae), trabecular separation and cortical thickness in three studied groups for coronal, middle and apical portions of socket showed significant differences in NgS powder and oil groups in comparison to control. Bone marrow star volume showed a significant variation between NgS powder and NgS oil in comparison to control, also bone marrow star volume showed decrease in it's value with increment in healing period in all groups. Trabecular number and width shows to be higher in NgS powder group in all portions of the socket in comparisum to control and NgS oil groups at 2 and 6 weeks duration. Blood vessels' number shows to be higher in middle portion in NgS powder group at 2 and 6 weeks duration in comparisum to NgS oil and control groups.

Conclusion: Nigella sativa powder suggest to be more effective and enhancer in acceleration of bone formation than oil.

KEYWORDS: Nigella sativa powder, extracted tooth , exodontia , periodontics , healing.

INTRODUCTION

Nigella sativa contains fifteen amino acids; these include eight of the nine essential amino acids required for a healthy diet. *Nigella sativa* oil may also contain parts of the essential oil,^(1,2) mostly thymoquinone, by which it acquires an aromatic flavor. However, different brands contain varying chemical compositions.⁽³⁾ Thymoquinone (TQ) is the predominant bioactive constituent present in black seed oil, it has potential in therapeutic strategy.⁽⁴⁾

MATERIALS & METHODS

The sample of the present study consists of twenty rabbits subjected to extract their upper two central incisors under general anesthesia. The left side filled once with Nigella sativa powder and once with Nigella sativa oil material, and the right side left for normal healing as a control group. The two sockets were sutured. The animals were grouped into: 1st group (10) rabbits, left socket of each rabbit filled by (0.068g) of Nigella sativa powder till 2/3 of socket length which had average length (2.3mm) after re-

moval of blood clot 2nd group (10) rabbits, left socket treated with Nigella sativa oil by using a piece of cotton impregnated in oil then inserted inside the socket till 2/3 of socket length after removal of blood clot and waiting for five minutes then removed it. Each group divided into two subgroups (5 in number) with healing periods; 2 weeks and 6 weeks and the socket examined histologically under light microscope.

Parameters Currently Used To Measure Trabecular Bone Microarchitecture

The microarchitecture descriptors are: trabecular width (Tb.Wid, in microns), trabecular number (or more exactly trabecular density) (Tb.N in per millimetre) and the trabecular separation (Tb.Sp, in microns), marrow space star volume(was calculated as V*m= $\pi/3$ × mean (*L*3), where *L* is the distance from a random point to the point intercepted by the trabecular bones lower V*m values indicate better trabecular bone connectivity). These parameters are derived from microscopic two dimension image measurements analyzer

software program (Vesterby *et al.* 1989). Measurement of trabecular bone microarchitecture by histomorphometric methods based on mathematical morphology and is calculated as the average of the all slices values. ⁽⁵⁾ Osteoblast, osteocyte, and blood vessles numbers calculated as the average of the all slices values for each period and for each group.

Statistical Analysis

All the data of the sample were subjected to computerized statistical analysis using SPSS version 15 (2006) computer program. The statistical analysis included:

Descriptive Statistics

- Mean
- Standard deviation (SD)

Inferential Statistics

• ANOVA test: for the comparison among the groups.

Least significant difference test LSD test: for variables that show significant differences among the study groups in ANOVA test.

RESULTS

Histomorphmetric analysis of bone architecture parameters in coronal, middle and apical portions of a socket of different studied groups in different periods illustrate the followings:

1. At 2 Weeks Duration

A. Coronal portion

According to the ANOVA (table 1), a highly significant difference was found in TBWID, TBSEP, CORWID and V while a significant difference was found in OBNO and OCNO only. Regarding to the LSD (table 2) for the mean of variables, a highly significant difference in TBWID, CORWID, OBNO and OCNO between NS powder in comparison with the control group and a highly significant difference in TBWID between NS powder and NS oil, The NS oil shows a significant difference in TBWID and COR-WID and a highly significant difference in OCNO in comparison with the control group. The control group found to have a highly significant difference in TB-SEP compared with NgS oil and powder; and a significant difference in V value in comparison with the NS powder. (Tables 1,2)

B. Middle portion

The ANOVA table (3-6) showed a highly significant difference in (TBWID, TBSEP, CORWID, OBNO and V); and a significant difference in BVNO only. According to the LSD table (3-7), the NgS

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powder shows a highly significant difference in TB-WID, CORWID and OBNO compared to the control group and a highly significant difference in OBNO and CORWID and BVNO in comparison with the NS oil. On the other hand, the NS oil found to have a significant difference in TBWID and CORWID; and a highly significant difference in OBNO compared with the control group while, in comparison with NgS powder, the NgS oil shows a highly significant difference in TBSEP and BVNO. Finally, the control group found to have a highly significant difference in TBSEP and V value compared with the both NgS oil and powder. (Tables 3,4)

C. Apical portion

According to the ANOVA table (3-8), a highly significant difference was found in all the variables except BVNO showed a no significant difference. The LSD table (3-9) shows a significant difference in TB-WID, CORWID, OBNO and OCNO between the NS powder and the control group, While the NgS powder shows a significant difference in TBWID CORWID, OBNO and OCNO when compared with the NS oil. The NS oil found to have a significant difference in TBNO compared with the control group. Finally, the control group revealed a highly significant difference in TBSEP and V when compared with both the NgS oil and powder. (Tables 5,6)

2. At 6 Weeks

A. Coronal portion

According to the ANOVA table (3-16), a highly significant difference was found in TBNO, TB-WID, TBSEP, CORWID and V. The LSD table (3-17) shows that the NgS powder had a highly significant difference in TBNO compared to the control group and a significant difference in TBNO also compared with NS oil, while a highly significant difference was found in TBWID and CORWID in comparison with the control and NgS oil. On the other hand, the NgS oil was found to have a significant difference in TB-WID compared to the control group and a highly significant difference in TBSEP and V compared to the NgS powder. Lastly, the control group shows a highly significant difference in TBSEP and V compared to the NgS oil and powder. (Tables 7,8)

B. Middle portion

Regarding the ANOVA table (3-18) shows a significant difference in TBNO and a highly significant difference in the rest variables were found Periodontics

among the groups. The LSD table (3-19) revealed that the NS powder had a significant difference in TBNO and a highly significant difference in TB-WID, CORWID, OBNO and OCNO compared to the control group and NgS oil, and a significant difference was found in BVNO compared to the control group and the NgS oil. The NgS oil shows a significant difference in CORWID compared to the control group and a highly significant difference in OBNO and V compared with the control and NgS powder respectively. Finally, the control group shows a highly significant difference in TB-SEP and V values compared to the NgS oil and

C. Apical portion

powder. (Tables 9,10)

According to the ANOVA table (3-20), a highly significant difference was found in all variables except in BVNO which was found with no significant difference. The LSD table (3-21) revealed that in the NgS powder there was a highly significant difference in TBNO, OBNO and OCNO in comparison with the control group and NgS oil, while it had a highly significant difference in TB-WID and CORWID when compared with the control group and a significant difference in CORWID only when compared with NgS oil. The NgS oil was found to have a significant difference in TB-WID and a highly significant difference in COR-WID compared to the control group. On the other hand, the control group shows a highly significant difference in TBSEP compared with the NgS oil and powder; and a significant difference in V value compared to the NgS oil and a highly significant difference in the same variable compared with the NgS powder. (Tables 11,12)

DISCUSSION

The effect of Nigella sativa seed extracts on varies body systems was studied *in vitro* and *in vivo*. The pharmacological investigation of the seed extracts reveal abroad spectrum of activities including immuneopotentiation, antihistaminic, antidiabetic, antihypertensive, antinflammatory, antitumor⁽⁶⁾ antiparasitic, antibacterial, antifungal and antioxidant.⁽⁷⁾ In recent study, the black seed oil extract induced bone healing as manifested by faster bone trabeculae formation and mature bone formation.⁽⁸⁾

Histomorphometric Findings:

The equality of means and variances of all parameters tested for most of micro architecture records between all contrasted study groups include experimental NgS (powder and oil) and control in all different portions (coronal,middle and apical) illustrate a high value in experimental than in control. This result can be explained on a fact that NgS has Thymoquinone (TQ) which is the predominant bioactive constituent present in black seed oil.⁽⁹⁾ It has potential in enhancement and recruitment of the progenitor cell to be differentiated to osteoblast cell (bone formative cell) and then trabecular bone formation.^(10,11)

Results conclude that there was a significant difference in bone architecture parameters in different intervals time. Increment in trabeculi width, trabeculi number and cortical width is shown in the NgS powder group in comparison to oil and control groups at 2 and 6 weeks duration in all tooth socket portions and it could be attributed to a fact that powder NgS will not lose any of its chemical component proteins, amino acid, aromatic oils, trace elements, vitamins and enzymes, essential fatty acids including omega 6 and omega 3 in comparisum to prepared oil NgS that records low volatile oils content.^(12,13)

Blood vessels number presented to be highly significant in middle portion for NgS powder group in comparisum to NgS oil and control groups, it could be explained into:^(14,15)

- 1. NgS itself enhance angiogenesis in area of bone formation but using of NgS in oil form and for 5 mints will not allow for complete absorption by surrounding tissue so it reflects on its effect when it compared with NgS powder.
- 2. The socket form of the anterior tooth of the rabbit is curved concerned with middle potion and therefore powder of NgS impacted in the middle and shows more effectiveness of its value than the oil one.

CONCLUSION

Nigella Sativa powder is seem to be more effective than oil one in the healing of extracted socket of rabbit as the results illustrate thicker trabecular width and more in number in all socket portions include coronal, middle and apical as well as the number of blood vessels in the middle portion exclusively.

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(Table 1) Comparison the coronal portions of (control, NgS oil and NgS powder within 2nd week)

ANOVA

	ΑΙγΟΥΑ	
Variables	F	Sig.
TBNO	1.357	.305
TBWID	31.500	.000
TBSEP	45.345	.000
CORWID	9.380	`.006
OBNO	6.796	.016
OCNO	8.000	.010
BVNO	3.800	.064
V	10.555	.004
	16 3	

df = 2

(Table 2) Multiple Comparisons LSD

Variable		Group	Mean Difference	Sig.
TBWID	1	2	75	.029
		3	-2.25	.000
	2	3	-1.50	.001
TBSEP	1	2	9.50	.000
		3	11.00	.000
	2	3	1.50	.262
CORWID	1	2	-2.75	.028
		3	-4.50	.002
	2	3	-1.75	.129
OBNO	1	2	-3.00	.065
		3	-5.25	.005
	2	3	-2.25	.150
OCNO	1	2	-2.00	.007
		3	-2.00	.007
	2	3	.00	1.000
V	1	2	.50	.990
		3	150.50	.003
	2	3	150.00	.003

* The mean difference is significant at the .05 level. (1=coronal control, 2=coronal oil, 3=coronal powder)

(Table 3) Comparison the middl	e portions of (control)	, NgS oil and NgS	powder within 2nd week
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Variables	F	Sig.
TBNO	2.000	.191
TBWID	11.382	.003
TBSEP	70.099	.000
CORWID	16.846	.001
OBNO	85.846	.000
OCNO	.661	.540
BVNO	7.824	.011
V	25.381	.000

Variable	Gre	oup	Mean Difference	Sig.
TBWID	1	2	-2.00	.017
		3	-3.25	.001
	2	3	-1.25	.102
TBSEP	1	2	5.50	.000
		3	11.75	.000
	2	3	6.25	.000
CORWID	1	2	-1.75	.041
		3	-4.25	.000
	2	3	-2.50	.008
OBNO	1	2	-8.50	.000
		3	-20.00	.000
	2	3	-11.50	.000
BVNO	1	2	-1.00	.265
		3	2.25	.025
	2	3	3.25	.004
V	1	2	127.50	.000
		3	162.50	.000
	2	3	35.00	.179

* The mean difference is significant at the .05 level. (1=Middle control, 2=Middle oil, 3=Middle powder)

(Table 5) Comparison the apical portions of (control, NgS oil and NgS powder within 2nd week)

ANOVA

Variables	F	Sig.
TBNO	8.591	.008
TBWID	9.632	.006
TBSEP	35.354	.000
CORWID	24.761	.000
OBNO	20.192	.000
OCNO	26.000	.000
BVNO	.000	1.000
V	31.707	.000

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(Table	6)	Multiple	Comparisons	LSD
1	10000	~/	minipie	0011101100110	

Variable		Group	Mean Differe	nce Sig.
TBNO	1	2	-2.25	.003
		3	-1.50	.024
	2	3	.75	.208
TBWID	1	2	-1.00	.083
		3	-2.25	.002
	2	3	-1.25	.038
TBSEP	1	2	3.50	.009
		3	8.75	.000
	2	3	5.25	.001
CORWID	1	2	-2.75	.019
		3	-6.75	.000
	2	3	-4.00	.002
OBNO	1	2	-1.25	.067
		3	-3.75	.000
	2	3	-2.50	.002
OCNO	1	2	-1.00	.117
		3	-4.00	.000
	2	3	-3.00	.001
V	1	2	125.00	.000
		3	175.00	.000
	2	3	50.00	.055

* The mean difference is significant at the .05 level. (1=Apical control, 2=Apical oil, 3=Apical powder)

(Tahle	7) AN	IOVA	test o	f coronal	portions in	(control N	S oil	and NoS	' nowder	within	6th week)
Inone	// 11	011	icsi 0	coronai	pornons in	(0011101, 112	50 011	unu ngo	powaer	<i>wuuuu</i>	0 week	1

Variables	F	Sig.
TBNO	9.176	.007
TBWID	33.876	.000
TBSEP	121.091	.000
CORWID	133.300	.000
OBNO	1.714	.234
OCNO	.339	.721
BVNO	.391	.687
V	59.303	.000

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(Tuble 0) Multiple Comparisons LSD

Variable	Gre	oup	Mean Difference	Sig.
TBNO	1	2	50	.330
		3	-2.00	.003
	2	3	-1.50	.013
TBWID	1	2	-6.25	.043
		3	-21.25	.000
	2	3	-15.00	.000
TBSEP	1	2	6.00	.000
		3	10.50	.000
	2	3	4.50	.000
CORWID	1	2	-3.00	.001
		3	-10.25	.000
	2	3	-7.25	.000
V	1	2	57.50	.001
		3	127.50	.000
	2	3	70.00	.000

* The mean difference is significant at the .05 level. (1=Apical control, 2=Apical oil, 3=Apical powder)

(Table 9) ANOVA test of middle portions in (control, NgS oil and NgS powder within 6th week)

Variables	F	Sig.
TBNO	5.727	.025
TBWID	51.233	.000
TBSEP	15.429	.001
CORWID	22.389	.000
OBNO	52.043	.000
OCNO	18.048	.001
BVNO	13.364	.002
V	25.038	.000
	df = 2	

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(Table	10)	Multiple	Comparisons	LSD
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Variable		Group	Mean Difference	Sig.
TBNO	1	2	.25	.538
		3	-1.00	.031
	2	3	-1.25	.011
TBWID	1	2	-1.75	.177
		3	-11.25	.000
	2	3	-9.50	.000
TBSEP	1	2	3.00	.005
		3	4.50	.000
	2	3	1.50	.102
CORWID	1	2	-2.25	.029
		3	-5.75	.000
	2	3	-3.50	.003
OBNO	1	2	-3.25	.000
		3	-5.75	.000
	2	3	-2.50	.002
OCNO	1	2	-1.75	.094
		3	-5.50	.000
	2	3	-3.75	.003
BVNO	1	2	75	.087
		3	-2.00	.001
	2	3	-1.25	.011
V	1	2	40.00	.009
		3	85.00	.000
	2	3	45.00	.005

* The mean difference is significant at the .05 level. (1=Apical control, 2=Apical oil, 3=Apical powder

(Table 11) Comparison the apical portions of (control, NgS oil and NgS powder within 6th week)

ANOVA

Variables	F	Sig.
TBNO	15.800	.001
TBWID	8.559	.008
TBSEP	10.048	.005
CORWID	29.207	.000
OBNO	38.613	.000
OCNO	33.091	.000
BVNO	.913	.435
V	8.866	.007

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Variable		Group	Mean Difference	Sig.	
TBNO	1	2	75	.135	
		3	-2.50	.000	
	2	3	-1.75	.004	
TBWID	1	2	-2.00	.017	
		3	-2.75	.003	
	2	3	75	.303	
TBSEP	1	2	3.50	.005	
		3	3.75	.003	
	2	3	.25	.795	
CORWID	1	2	-5.50	.001	
		3	-8.25	.000	
	2	3	-2.75	.034	
OBNO	1	2	-1.25	.089	
		3	-5.50	.000	
	2	3	-4.25	.000	
OCNO	1	2	50	.479	
		3	-5.00	.000	
	2	3	-4.50	.000	
V	1	2	45.00	.038	
		3	77.50	.002	
	2	3	32.50	.113	

(Table 12) Multiple Comparisons LSD

* The mean difference is significant at the .05 level.

(1=Apical control, 2=Apical oil, 3=Apical powder)

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Periodontal Health Status & Salivary Elements Analysis (Iron & Potassium) Among Group of Patients with Rheumatoid Arthritis & Chronic Periodontitis

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ABSTRACT

Background: Periodontal diseases are common in the society & some researchers suggested an association between rheumatoid arthritis (RA) & periodontal diseases. The aim of the present study was to determine the periodontal health status in patient with RA & chronic periodontitis & compare it with those having chronic periodontitis only & determine the level of salivary elements; iron (Fe) & potassium (k) in both groups & compare it with control group & correlate between these salivary elements with the periodontal parameters plaque index (PLI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD) & clinical attachment level (CAL).

Materials & Methods: The samples were recruited from patients referred to department of rheumatology at Baghdad Hospital. Seventy five (75) patients participated in this study, twenty five of them had rheumatoid arthritis with chronic periodontitis; twenty five had chronic periodontitis only without arthritis & another twenty five patients were periodontally & systemically healthy (control group). The patients were with age range 40-50 years with no other systemic disease. Periodontal parameters were measured in all groups at four surfaces which include plaque index, gingival index, bleeding on probing, probing pocket depth & clinical attachment level. Salivary samples were collected under standardized condition & then analyzed for estimation of the level of potassium by using flame atomic absorption spectrophotometry (AAS) while the iron level by using spectrophotometric analysis.

Results: Patients with RA & chronic periodontitis had higher prevalence of site presenting dental, plaque, a higher rate of gingival inflammation & bleeding on probing, greater probing depth & clinical attachment level with a significant difference from the group of patients with chronic periodontitis alone without RA the results also revealed a higher concentration of iron & potassium among the rheumatoid arthritis group than the second & control group with a statistically highly significant difference between the three groups.

Conclusion: The results suggest higher potentiality for moderate to severe periodontitis involvement among RA patients with higher levels of salivary iron & potassium. The coexistence of RA & chronic periodontitis could possibly influence the inflammatory process & the pathogenesis of one disease on the other.

KEYWORDS: Rheumatoid arthritis, Chronic periodontitis, Salivary elements.

INTRODUCTION

Periodontitis is an inflammatory disease of tooth supportive tissues & is characterized by destruction in periodontal ligaments & alveolar bone besides pocket formation & gingival recession.⁽¹⁾ Rheumatoid arthritis (RA) is thought to be an auto immune disease that affects several organs & systems & it is also associated with destruction of joint connective tissue & bone.⁽²⁾ It is a chronic destructive inflammatory disease characterized by the accumulation & persistence of an inflammatory infiltrate in the synovial membrane that leads to synovitis & the destruction of the joint architecture, deformity &loss of function (Figures 1,2).



(Figures 1&2) structural deformity of the hand joints. Permanent deformity is an unwanted result of the inflammatory process

An association between periodontitis & RA has been considered since 1820s. Both diseases are chronic &may present with bursts of disease activity. There is the possibility of a common genetic trait predisposing to both conditions (dysregulation of the inflammatory mechanisms). Porphyromonas gingivalis is a common pathogen in periodontal infection & it has also been identified in synovial fluid. This periodontal pathogen may carry a unique risk for development of autoimmune antibodies associated with RA.⁽⁴⁾

Both periodontitis & RA present an imbalance between pro-inflammatory & anti-inflammatory cytokines which is responsible for the tissue damage. In this sense, both conditions are associated with

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destruction of bones, mediated by inflammatory cytokines such as inter leukin-1, tumor necrosis factor-α & prostaglandin E2.^(2,5) A bidirectional relationship of RA & periodontitis may involve RA affecting the pathogenesis of periodontitis & vice versa.⁽⁶⁾

Periodontitis might interfere with the pathogenesis of RA through bacteremia, presence of inflammatory mediators, bacterial antigens & immunoglobulins in the serum, while RA may influence the pathogenesis of periodontitis through its motor & emotional impairment. Motor impairment may make it more difficult to perform adequate oral hygiene. The salivary flow reduction due to medication or secondary Sjogren syndrome may increase supra gingival plaque formation in these individuals. Also psychological alterations found among RA patients were suggested as risk indicators for periodontitis.^(7,8)

Iron, Potassium & RA:

Iron is an essential element for nearly all living organisms by participating in a wide variety of important metabolic processes, such as oxygen transport (binding & release of hemoglobin), DNA synthesis, electron transport, lipid metabolism, photosynthesis & gene regulation.^(9,10) The role of iron in patient with RA had been studied by spectrometric analysis & showed elevated concentration of iron in the synovial fluid of RA patients than normal subjects ⁽¹¹⁾. Also it has been found that patient with RA had low levels of iron in the serum which will lead to development of anemia termed as anemia of inflammation (AI).⁽¹²⁾

Potassium is a very important mineral for the proper function of all cells, tissues & organs in the human body. It is also an electrolyte that conducts electricity in the body. It is the major intra cellular ion that is crucial to heart function & plays a key role in skeletal & smooth muscle contraction. Also it has been suggested that increasing consumption of foods rich in potassium may play a role in osteoporosis prevention particularly among elderly women.^(13,14) It has been found that patients with RA have lower salivary & serum potassium concentration than healthy subjects.⁽¹⁵⁾ According to the National health & Nutrition Survey –III, serum of the patients with RA have been tested for potassium & they found 18% appeared to be in the normal range.⁽¹⁶⁾

According to our knowledge there is no study that determine the concentration of iron & potassium in the saliva of patients having both rheumatoid arthritis & chronic periodontitis, so it was decided to conduct this study to determine the periodontal health status of those patients & measure the concentration of these two salivary elements & correlated with the periodontal parameters (PLI, GI, BOP, PPD & CAL).

MATERIALS & METHODS

Human sample: subjects included in the study were drawn from patients attending the department of Rheumatology & dental department at Baghdad hospital from the period between June to September 2011. The sample is composed of 75 patients. The inclusion criteria for the total sample were: both genders, age between 40-50 years, must have not less than 4 periodontal sites with pocket depth of 4mm or greater, had good general health with no history of systemic disease (except RA) & had normal weight & length according to Body Mass Index (BMI) which is defined as weight in kilogram divided by the square of height in meters & its normal value is 18.5-25 because increase or decrease in weight may be a risk factor of RA. The exclusion criteria included: smoking, patients who undergone periodontal treatment &/ or antibiotic therapy for the last 3 months, females with pregnancy, breast feeding, postmenopausal status & contraceptive pills intake.

Design of the Study:

All the individuals were informed of the purposes of the investigation. A questionnaire was designed & it include name, age, sex, medical & dental histories, use of medication & smoking. The sample was divided into 3 groups:

- 1. The first group was 25 patients (3 males & 22 females) diagnosed to have RA & chronic periodontitis. The diagnosis of RA was done according to the Revised criteria for the classification of RA of the American College of Rheumatology 1987,⁽¹⁷⁾ also according to the laboratory investigation (ESR, Latex test). It was the first time for the patient to be diagnosed as having RA. The criteria for classification of RA include the following:
 - Morning stiffness in & around Joints lasting at least 1 hour before maximal improvement
 - Soft tissue swelling (arthritis) of 3 or more Joints areas observed by a physician
 - Swelling (arthritis) of the proximal interphalangeal, metacarpophalangeal or wrist joints
 - Symmetric swelling (arthritis)
 - Rheumatoid nodules
 - The presence of Rheumatoid factor
 - Radiographic erosions &/or periarticular osteopenia in hand &/or wrist joints

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Criteria 1 through 4 must have been present for at least 6 weeks. RA is defined by the presence of 4 or more criteria.

It is important to mention for future researches that a new criterion of classification of RA had been developed in 2010.⁽¹⁸⁾

This new classification system redefines the current paradigm of RA by focusing at earlier stages of disease that are associated with persistent &/or erosive disease, rather than defining the disease by its late-stage features. This will refocus attention on the important need for earlier diagnosis of the disease to prevent or minimize the occurrence of the undesirable sequelae of the disease.⁽¹⁸⁾

- 2. The second group of the sample was 25 patients that had chronic periodontitis without RA. The patients had at least 4 sites with probing depth of 4mm or greater with clinical attachment loss of 1-2mm or greater.
- 3. The third group was another 25 patients with healthy periodontium & systemically healthy. The three groups were uniform with regard to age & sex. It is important to mention that women are more affected with RA than men in a ratio of 3:1 & has a peak incidence of onset in women in the fourth & fifth decades of life,⁽¹⁹⁾ so our sample had more females than males (4 males & 22 females).

Collection of Saliva: Unstimulated salivary sample was collected. The patients were asked not to eat or drink except water one hour before collection & sited in a relaxed position. The saliva was collected in the floor of the mouth & then the patient let it drool passively over the lower lip into a cylinder until 5ml was collected. Then salivary samples were taken to the laboratory of poisoning center in the specialized surgery hospital, & centrifuged at 4000 rpm for 15min. the clear supernatant was separated by micropipette & stored at (-20Co) in a deep freeze till being assessed.

Clinical Periodontal Examination: All the patients underwent an oral examination by an experienced periodontist. The periodontal parameters that had been calibrated include:

- 1. PLI of Silness & Loe 1964.(20)
- 2. GI of Loe & Silness 1967.⁽²¹⁾
- 3. Bleeding on probing (BOP): a blunt periodontal probe was inserted to the bottom of periodontal pocket. If bleeding occur within 30 sec. after probing, the site was given a positive score (1) & a negative score (0) for the non bleeding site.⁽²²⁾

- 4. Probing pocket depth (PPD): The distance from gingival margin to the most apical penetration of the periodontal probe was recorded.
- 5. Clinical attachment level (CAL): was calculated by adding the values of probing depth & the distance between the cement enamel junction (CEJ) & the gingival margin (GM), when the gingival margin was located apically to the CEJ. When the GM was located coronal to the CEJ, the value of the CEJ-GM distance was subtracted from the value of probing depth.

Biochemical Analysis: Frozen salivary samples were allowed to thaw & come to room temperature. The salivary elements F & K were analyzed at the poisoning consultation center/surgical specialty hospital. Potassium ion determined using flame atomic absorption spectrophotometer (Buck scientific, 210 VGP, USA) procedure by air-acetylene. The concentration level was expressed as (mmol/L) unit.

The method used to determine the level of salivary iron was by colorimetric method. A readymade kit (Linear chemicals-Spain) was used according to the manufacturer instruction. The intensity of the color is proportional to the amount of iron in the sample & it was read at a wave length of 560 nm. The concentration level was expressed as μ mol/L unit.

Statistical analysis: The data were processed & analyzed using the statistics package for social science SPSS Inc., version 17 for windows XP & excel 2007. Both descriptive & inferential statistics were used. *t*-test, chi-square, anova tests were used where indicated level of significance was 0.05.

RESULTS

Seventy five patients were involved in this study & were subdivided as follows:

- 25 patients with rheumatoid arthritis & chronic periodontitis (RA group)
- 25 patients with chronic periodontitis only (CP group)
- 25 patients were periodontally & systemically healthy (control group).
- Table 1 showed that the mean PLI in RA group (1.837) was significantly higher than the CP group (1.149).
- Table 2 showed that the mean GI in RA group (1.319) was significantly higher than CP group (1.141).
- Table 3 showed than number & percentage of bleeding sites in RA group which was higher (70.1%) than CP group (55.8%) with significant difference.
- Mean PPD & CAL among RA group was found higher than CP group with a highly significant dif-

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ference as seen in tables 4&5.

Regarding the K ion concentration among the three groups, it was found that the mean level of K ion in both RA group & CP group was higher than the control group expressed in μ mol/c (table 6). Inter group comparison of K level concentration revealed a highly significant difference between different groups (table 7). Also comparison between the three groups by applying F test revealed a highly significant difference (table 8).

The mean concentration of salivary iron in RA group was found much higher than both CP & control group expressed in μ mol/c (table 9). Inter group comparison revealed a highly significant difference between different groups (table 10) & by applying *(Table 1) Mean and SD of Pl among groupl and group ll*

F test the comparison between the three groups was found highly significant (table 11). The correlations between clinical periodontal parameters & salivary constituents K & iron among RA & CP groups were shown in table 12. In this table the correlations between K & iron levels & plaque index in both groups were mostly weak in negative direction, while the correlations with GI were varies between weak not significant positive & negative directions.

The only significant strong negative correlation was found between the level of salivary ZK ion among RA group & bleeding on probing (-0.608).

Negative & positive weak non significant correlations were also found between K & iron levels of both groups with PPD & CAL

Groups	Mean	SD	t-test	P-value	Sig
Groupl	1.837	0.255	7.981	0.001	HS
Groupll	1.149	0.113			

P<0.01 highly significant

(Table 2) Mean and SD of Gl among groupl and group ll

Groups	Mean	SD	t-test	P-value	Sig
Groupl	1.319	0.076	8.641	0.002	HS
Groupll	1.141	0.069			

(Table 3) Number and percentage of bleeding sites among groupl and group ll

Scores of	Gro	up l	Gro	up ll	Chi-square	P-value	Sig
BOP	No.	%	No.	%			
0	582	29.9	828	44.2	79.81	0.049	S
1	1364	70.1	1044	55.8			

*P<0.05 Significant

(Table 4) Mean and SD of PPD among groupl and group ll

Groups	Mean	SD	t-test	P-value	Sig
Groupl	6.065	0.547	12.146	0.028	S
Groupll	4.296	0.479			

(Table 5) Mean and SD of CAL among groupl and group ll

Groups	Mean	SD	t-test	P-value	Sig
Groupl	4.472	0.408	5.618	0.007	HS
Groupll	3.684	0.569			

(Table 6) Mean and SD of K ion concentration among the three groups

Groups	Mean(mmol/L)	SD
Groupl	11.424	2.06
Groupll	10.553	1.992
GroupIII	9.112	1.368

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Table (7) Inter group comparison of K level concentration

Groups	t-test	P-value	Sig			
Groupl&groupll	6.239	P<0.01	HS			
Groupl&grouplll	4.596	P>0.01	HS			
Groupll&grouplll	6.501	P<0.01	HS			
(Table 8) Comparison between	Table 0) Companies between the three energy according the View concentration					

(Table 8) Comparison between the three groups regarding the K ion concentration

Groups	ANOVA F-test	P-value	Sig
Groupl,ll,lll	40.183	P<0.01	HS

(Table 9) Mean and SD of Fe ion concentration among the three groups

Groups	Mean(µmol/L)	SD
Groupl	9.504	3.428
Groupll	5.913	3.559
GroupIII	2.80	1.104

(Table 10) Inter group comparison of Fe level concentration

Groups	t-test	P-value	Sig
Groupl&groupll	2.478	0.021	S
Groupl&grouplll	6.096	P<0.01	HS
Groupll&grouplll	4.159	P<0.01	HS

(Table 11) Comparison between the three groups regarding the Fe concentration

Groups	ANOVA F-test	P-value	Sig
Groupl,ll,lll	19.97	P<0.01	HS

(Table 12) Correlation between clinical periodontal parameters and biochemical parameters

			Pl	Gl	BOP	PPD	CAL
Group l	K	r	-0.262	0.123	-0.608	0.088	-0.213
		р	0.206	0.557	0.001	0.676	0.307
	Fe	r	-0.178	-0.138	-0.246	-0.257	0.193
		р	0.396	0.510	0.235	0.214	0.350
Group ll	K	r	-0.303	-0.101	-0.142	-0.268	0.125
		р	0.140	0.630	0.497	0.196	0.552
	Fe	r	-0.151	0.181	0.068	-0.117	0.193
		р	0.471	0.387	0.748	0.578	0.354

DISCUSSION

Periodontal disease & its mechanism of inflammatory reactions result in the destruction of tissue & bone in a pattern similar to that which mediate destruction of soft tissue & erosion of bone in rheumatoid arthritis. In both conditions a persistent inflammatory reaction occurs in areas composed of connective tissue & bone with the activation of complement, production of cytokines & release of other inflammatory cell products.^(6,23) The literature regarding the relationship between periodontal disease & rheumatoid arthritis is controversial. The methodologies applied in the studies are as diverse as their results & conclusions.⁽⁷⁾ When compared saliva constituents with other studies, one must keep in mind differences in age groups, type of saliva collected (stimulated or non-stimulated), way of collection, flow rate, diet that may affect composition of saliva, the variation in sampling procedure as well as technique of analysis which may

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explain the differences in the result of the studies.⁽²⁴⁾

In this study & regarding the periodontal health status of the two main groups which were the RA group & CP group, it was found that both mean plaque & gingival indices & the number & percentage of bleeding sites were significantly higher in RA group than CP group & this could be attributed to the stiffness of hand muscles & inability of the patient to remove all dental plaque which is the main causative factor of gingival inflammation, so this reduction in hands muscle function lead to difficulty in performing adequate oral hygiene. Moreover, interesting observations regarding the complexity of the oral & systemic challenge provide unique mechanisms by which dysregulation of host responses could occur.^(7,25) The higher levels of periodontal inflammation in RA patient group than other groups are in conformity with Mirrielees 2010.⁽²⁶⁾

The mean values of PPD & CAL in RA group were significantly higher compared to CP group & this could be related to local & systemic factors. The local factor is dental plaque & the systemic factor is the defect in the immune system which could result in inflammatory-mediated destruction predisposing them to periodontitis due to an unbalanced cytokine expression profile.^(2,7) Regarding the salivary concentration of K ion & iron in the RA group which had at the same time chronic periodontitis. We could not find a similar study which measures the concentration of these two elements in the saliva of patients having both these diseases to compare with it. The researchers either measure the levels of these elements in one of these diseases or measure them in the serum & not saliva. However, in this study, it was found that the mean K ion concentration was higher in RA group than the chronic periodontitis group & the control group. This result disagrees with the study done by Rastmanesh R 2009⁽¹⁵⁾ who found lower salivary & serum K concentration in patients with RA. An explanation for this difference is that in the previous study, the patients had only RA without chronic periodontitis. So the coexistence of RA & periodontitis would offer an interesting opportunity to study the possible influence of periodontal inflammatory process on RA progression. Regarding the salivary iron concentration of patients with RA & CP, it was found higher than the second group & higher than the control group. In a possible explanation for this result, we have to know the following: during inflammation, T cells & macrophages produce a number of cytokines which influence the metabolism of iron, affecting its cellular uptake, transport, storage as well as its absorption.⁽²⁷⁾ This will result in the development of anemia of inflammation (AI) which is most common condition in patients with rheumatic disease & it is characterized by low to normal serum iron levels, low serum iron binding capacity & normal to elevated ferritin concentrations (Ferritin is iron storage protein).⁽¹¹⁾ Transferrin which is the iron transport protein have the function of controlling the level of iron in biological fluids, it allow ferric iron to remain soluble & facilitates the cellular import of iron.⁽²⁸⁾ Transferrin has an extremely high affinity for binding iron, it contain two specific high affinity iron binding sites. Transferrin was found localized within the cells of striated ducts, in some intercalated ducts as well as in serous acinar cells of salivary glands of patients with RA, & because transferrin has a high affinity for iron, this could explain increase iron in saliva of patient with RA.⁽²⁹⁾

CONCLUSION

In conclusion, patients with RA have a higher level of periodontal inflammation, higher levels of salivary iron & potassium than patients without RA.

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Oral Health Status in Relation to Salivary Iga and Antioxidants among a Group of Gasoline Workers Stations

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ABSTRACT

Background: Workers of gasoline stations have the potential to be exposed to hazardous substances that are present in the occupational environment like gasoline vapors which is carcinogenic to the humans. The aim of this study was to evaluate salivary IgA and salivary antioxidants and the relationship of these variables to oral health status among workers in gasoline stations.

Materials and methods: 44 males aged (33-39 year) were enrolled in this study, 23 of them were workers in gasoline station for at least five years and 21 healthy males were control group matching the workers in age. Stimulated saliva was collected and subjected to biochemical analysis for detection of salivary IgA and antioxidants (uric acid, magnesium and zinc ions). Oral health status was evaluated by using decayed, missing and filled surface index (DMFS), plaque index (PII), gingival index (GI), calculus index (Call) and periodontal pocket depth (PPD).

Results: Salivary IgA was lower for the study group compared to the control group with highly significant difference(P<0.01)while uric acid was significantly higher for study group than the control one(P<0.05).Similarly magnesium level was higher among the workers than the control group though the difference was not significant(P>0.05).In contrast the level of salivary zinc was higher for the control group than the study group with no significant difference. Regarding oral health status, DMFS, PII, GI, Call and PPD index were higher for study group compared with control group although the differences were not significant except for Call which was highly significant (P<0.01). In general, not significant correlation were found between salivary variables and the other oral parameters for the two groups.

Conclusions: Gasoline workers stations were associated with decrease salivary IgA and increase salivary antioxidant (except zinc) this may explain the increase in caries experience and periodontal diseases among those workers.

KEYWORDS: Gasoline workers, Oral health, IgA, Antioxidant....

INTRODUCTION

Millions of workers in a variety of occupations were found to be exposed to hazardous substances which can be present in the form of gases, vapors, mist, fumes or particles, so diseases in some of these workers can be attributed to the exposure to these substances.^(1,2) Gasoline is a refined product of petroleum consisting of a complex mixture of chemicals including benzene which is well-known in its carcinogenicity to human.⁽²⁾ Benzene and its metabolites seem to be genotoxic to humans, causing primarily chromosomal aberrations.⁽³⁾ Routs of exposure can be take place through inhalation, dermal absorption or ingestion.⁽¹⁾

Salivary IgA is the first line of defense against microbial invasion.⁽⁴⁾ It is present in high concentration in saliva which represent the summation of IgA of salivary glands in addition to serum IgA from gingival fluid.⁽⁵⁾ Similarly antioxidant system is one of the important defense mechanism in saliva. An antioxidant is a molecule capable of inhibiting the oxidation reactions which can produce free radicals. In turn, these radicals can induce damage to the cells.⁽⁶⁾ Antioxidant system includes enzymatic and non-enzymatic antioxidants, uric acid is one of the non-enzymatic antioxidant in human saliva.⁽⁷⁾ It is a relatively powerful scavenging antioxidant of water soluble free radicals.⁽⁸⁾ On the other hand enzymatic antioxidants require a variety of trace elements for their activation like zinc and magnesium.⁽⁹⁾ In addition, it has been found that magnesium is involved in the antioxidant defense mechanisms of the body⁽¹⁰⁾ and its deficiency was found to induce elevation in the formation of oxygen radicals.⁽¹¹⁾

Extensive studies are needed to evaluate biological damages at different levels and to decrease the risk for serious diseases among workers in gasoline stations. Since no previous Iraqi study concerning this subject, this study was carried out to investigate the oral health condition in relation to salivary IgA and antioxidants among gasoline workers stations.

SUBJECTS, MATERIALS & METHODS

Forty four males aged (32-38) were enrolled in this investigation, study group composed of 23 workers in gasoline stations in Baghdad city for at least five years and control group included 21 males matching the study group by age. All individuals should be non-smokers, with no chronic medical illness and shouldn't take any medications to exclude any factor that may affect the parameters examined in this study. Stimulated salivary samples were collected following assessment of dental plaque under standard condition according to the instructions cited by Tenovuo and Lagerlof.⁽¹²⁾ Salivary samples were taken to the laboratory for biochemical analysis. Immunoglobulin A in saliva was determined by radial immunodiffusion technique of Mancini et al.⁽¹³⁾ Salivary antioxidant were determined by using ready kit

(BioMerieux sa, France) for uric acid while salivary magnesium and zinc ions were measured by using atomic absorption spectrophotometer flame (Model Buck 210 VGP USA).

Caries experience was assessed by the application of decayed, missing and filled surface index⁽¹⁴⁾ (D1-2 MFS). Plaque index⁽¹⁵⁾ (PII) and calculus index⁽¹⁶⁾ (CalI) were used for recording oral cleanliness. Periodontal status evaluated by using gingival index⁽¹⁷⁾ (GI) and periodontal poket depth⁽¹⁸⁾ (PPD). Data analysis was conducted through the application of the SPSS (version 16).Student's-test and Pearson's correlation coefficient was applied. The confidence limit was accepted at 95% (P<0.05).

RESULTS

Table 1 revealed that the salivary IgA was lower among study group compared with control group with highly significant difference (P<0.01). On the other hand uric acid showed higher value among study group with highly significant difference(P<0.01). Although salivary magnesium was higher among study group but the difference was not significant (P>0.05) also no significant difference was found regarding salivary zinc (P>0.05) in spite it was higher among control group. Table 2 illustrates that DMFS, D, M and F values were higher among the study group but the differences were statistically not significant (P>0.05). Results also showed that PII, GI, CalI and PPD were higher among the study group with no significant differences (P>0.05) except for Call which was highly significant difference (P<0.01) (Table 3). As shown in Table 4 no significant correlations were recorded between caries experience with salivary variables in various directions for the two groups. Similarly no significant correlations were found between salivary variables and severity of dental caries in various directions except for IgA and D2 among the study group which was highly significant correlation (Table 5). Regarding oral cleanliness and periodontal health, Table 6 demonstrates no significant correlations between PII, GI, CAL and PPD with salivary variables in various directions for the study and control group.

DISCUSSION

Exposure to toxic agents can result from natural and environmental factors, occupational environment or industrial accidents.⁽¹⁹⁾ Gasoline station workers have increasing the risk for developing serious diseases due to the possible long-term effects of the petroleum derivatives on their health.⁽²⁰⁾

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Saliva is a unique fluid,as it is the first biological medium confronted by external materials that are taken into the body as parts of food, drink or inhaled volatile ingredients⁽²¹⁾ also saliva used to study the influence of environmental factors on immunological parameters.⁽²²⁾ In the present study salivary IgA was lower among study group than the control group with highly significant difference. This could be attributed to that salivary IgA is often decreased after chemical exposure.^(22,23) This finding may explain the low defense of a given individual against external intruders. ⁽²⁴⁾ In addition decreased concentration of secretory IgA is a potential indicator of increased disease risk.⁽²⁵⁾

Concerning antioxidant, uric acid was significantly higher among study group than control group also magnesium level was higher among the study group though the difference was not significant. This may be related to the fact that the body raises the level of its antioxidant system to combat the oxidative damage,⁽²⁶⁾ as the functions of antioxidant are to prevent the generation of free radicals and to inactivate them after generation.⁽²⁷⁾ Level of salivary zinc ion was lower among the study group but statistically the difference was not significant. Zinc regarded as one of the main healing minerals, it is essential for the activity of over 300 enzymes in the body. In addition it forms part of the enzyme (carbonic anhydrase) and functions as antioxidant.⁽²⁸⁾ Previous studies have shown that constituent of the gasoline fumes could be carcinogenic^(2,29) by producing free radicals which reacts with trace elements and in turn affects the total antioxidant status of the individual. In addition it was found that decrease in plasma levels of zinc probably as a result of interference in their metabolic pathway of the exposed groups,⁽³⁰⁾ this may explain the low level of zinc among the study group.

In regard to dental caries, the current study demonstrated a higher caries experience for the study group than the control one although the differences were not significant. This may be due to the lower level of salivary IgA among study group. On the other hand no significant correlation were recorded between salivary IgA and caries experience except with DS which was significant negative correlation among the study group only, this may explain the higher caries experience among them as salivary IgA consider as the first line of defense against microbial invasion⁽³¹⁾ and increased its level in saliva can enhance the elimination of Streptococcus mutans from oral cavity and interfere with its cariogenicity,⁽³²⁾ this also may be the cause of highly significant correlation between IgA and D2 among the study group. In addition no significant correlations were recorded between an-

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tioxidants (uric acid, magnesium and zinc) and caries experience in both groups, the negative correlations between magnesium with DMFS and DS may be attributed to that magnesium level was inversely associated with caries experience.⁽³³⁾

The results also showed higher PII, GI and PPD for study than control group in spite that the differences were not significant. This may be attributed to the lower level of salivary IgA among the study group also no significant correlation were recorded between salivary IgA and PII, GI, Cal and PPD in both groups. Salivary IgA has antibacterial properties that can protect against oral infections⁽³⁴⁾ and this may explain the negative correlation between IgA and gingival index. Furthermore, It was documented that different salivary antioxidant provide protection against radicals-induced damage of periodontal tissues.⁽³⁵⁾ This may explain the non significant differences for PII, GI, and PPD between study and control group although they were higher among the study group. While other studies found that the level of salivary uric acid⁽³⁶⁾ and magnesium⁽³⁷⁾ were decrease in patients with severe periodontitis. On the other hand, calculus accumulation was higher among study group with highly significant difference, this may give another explanation for the higher plaque, gingival and periodontal pocket depth indices among gasoline stations workers because dental calculus plays a role in periodontal disease pathogenesis since it is a mineralized dental plaque and it acts as a retentive factor for dental plaque.⁽³⁸⁾

Results of this study showed that gasoline workers stations belong to a risk group for public agencies concerned with environmental quality and public health to applied educational and preventive programs concerning those populations.

Variable (mg/dl)	Study group	Control group	Statis	tics
	Mean ±SD	Mean ±SD	t-value	Р
IgA	37.01 1.05	39.09 2.30	3.79	0.00**
Uric acid	2.17 0.90	1.03 0.58	4.91	0.02*
Magnesium	1.45 0.80	0.55 0.28	4.86	0.12
Zinc	11.56 4.21	15.17 3.55	-3.05	0.31

(Table 1) Salivary variables among study and control group

* Significant(P<0.05) ** Highly significant(P<0.01) d.f.=42

(Table 2) Caries -experience among study and control group

Caries experience	Study group		Control group		Statistics	
	Mean ±SD		Mean ±SD		t-value	Р
D	10.21 5.12		5.14 4.46		3.48	0.95
М	15.00	13.56	8.28	7.43	2.00	0.06
F	6.26 10.77		6.23 7.68		0.00	0.24
DMFS	31.47 18.37		20.38	13.01	2.29	0.11

d.f.=42

(Table 3) PlI, GI, CalI and PPD among study and control group

Variable (mg/dl)	Study group	Control group	Statistics	
	Mean ±SD	Mean ±SD	t-value	Р
PII	1.36 0.50	0.86 0.44	3.40	0.32
GI	0.95 0.51	0.64 0.36	2.31	0.11
Call	0.53 0.51	0.14 0.16	3.23	0.00**
PPD	1.86 0.71	1.49 0.33	2.15	0.10

** *Highly significant*(*P*<0.01) *d.f.*=42

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(Table 4) Correlation coefficient between salivary variables and caries experience

Variable		Study	group	Control group					
	DN	DMFS		DS		DMFS		DS	
	r	Р	r	Р	r	Р	r	Р	
IgA	-0.10	0.64	-0.41	0.04*	0.23	0.31	-0.14	0.53	
Uric acid	0.05	0.82	0.24	0.28	0.19	0.64	0.17	0.47	
Magnesium	-0.25	0.26	-0.08	0.71	-0.14	0.54	0.00	1.00	
Zinc	-0.22	0.31	0.16	0.60	0.13	0.56	0.01	0.97	

* Significant(P<0.05)

(Table 5) Correlation coefficients between salivary variables and severity of dental caries

Groups	Dental	IgA		Uric acid	Uric acid		Magnesium		Zinc	
	caries	r	Р	r	Р	r	Р	r	Р	
Study	D1	0.07	0.73	0.04	0.86	-0.11	0.61	0.20	0.36	
group	D2	-0.51	0.01**	0.31	0.15	-0.16	0.46	-0.06	0.78	
	D3	-0.33	0.11	0.23	0.29	-0.12	0.58	0.02	0.89	
	D4	0.06	0.75	-0.15	0.47	0.23	0.29	0.06	0.78	
Control	D1	-0.19	0.39	0.00	0.98	0.07	0.76	-0.19	0.39	
group	D2	-0.01	0.94	0.11	0.63	-0.09	0.70	0.02	0.99	
	D3	-0.23	0.30	0.01	0.95	0.04	0.86	0.16	0.46	
	D4	0.02	0.92	0.47	0.31	-0.00	0.98	0.20	0.38	

** Highly significant(P<0.01)

(Table 6) Correlation coefficient between salivary variables and PII, GI, CalI and PPD

Groups	Variable	IgA		Uric acid	l	Magnesi	um	Zinc	
		r	Р	r	Р	r	Р	r	Р
Study	PlI	0.00	0.97	-0.40	0.6	0.23	0.29	0.17	0.43
group	GI	-0.01	0.95	0.06	0.78	0.16	0.46	0.18	0.40
	CalI	-0.03	-0.89	-0.29	0.17	0.07	0.76	0.15	0.49
	PPD	0.07	0.74	0.08	0.72	-0.12	0.58	0.10	0.65
Control	PlI	0.10	0.64	0.17	0.48	-0.11	0.63	-0.02	0.92
group	GI	0.17	0.44	-0.25	0.27	-0.29	0.21	-0.23	0.31
	Call	-0.03	0.88	-0.03	0.90	-0.02	0.91	0.12	0.58
	PPD	0.13	0.55	-0.44	0.40	-0.56	0.80	0.05	0.88

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Oral Diagnosis

Cyst of the Jaw Bones

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ABSTRACT

Background: Cysts of the jaw bones are more common than in any other bone in the body. A total of 5356 biopsies were studied of which 418 (7.804%) werecysts involving the oral region. Odontogenic cyst comprised 348 cases (83.25%). The most common odontogenic cyst was periapical cyst forming 31.25% and keratocyst came second with an incidence of 16.03% of the total cystic lesions examined.

Materials and methods: The sample of study composed of 418 biopsies which had been diagnosed as being oral cysts. Age, sex, site of lesion and final diagnosis were obtained from the files of the patients.

Results: Out of 418 cysts, 83.25%, were diagnosed as odontogenic cysts while nonodontogenic cysts comprised 3.34% of the total number. Periapical cyst was the most common cystic lesion forming 39.39% of the total number, with most cases occurring in the maxilla (23.3%). Next come odontogenickeratocyst (19.25%), followed by dentigerous cyst (14.94%).

epidermoid cyst was the most predominant nonodontogenic cyst (0.96%). The highest incidence of cystic lesions occurred at the age of 21-30 (31.10%) with male predominancy.

KEYWORDS: Oral cysts, Periapical cyst, Keratocyst.

INTRODUCTION

A true cyst forms when developmental or inflammatory factors stimulate proliferation of epithelial cells surrounding a tooth. As these cells grow, the central cells become removed from their nutrient supplied by adjacent vascular connective tissue and become necrotic. Subsequently, an epithelium-lined cavity or sac is formed.⁽¹⁾ So simply, by definition, a cyst is a pathological, often fluid filled cavity lined by epithelium.⁽²⁾

The dental practitioner is presented with a variety of cystic lesions throughout his professional life. Cysts of the jaw bones are more common than in any other bone in the body.⁽³⁾ Epithelial lined cysts are seen only in the jaw bones with rare exceptions. This epithelial lining is mostly odontogenic in origin.

A few cysts may be lined by respiratory epithelium originating from the lining of the maxillary sinus, such as the nasopalatine duct cyst and other fissural cysts.Non-epitheliated bone cysts are occasionally seen in the jaws especially the mandible. As for the soft tissue cysts, these are uncommon lesions, but may be seen sometimes.⁽⁴⁾

The classification of cysts was recommended by the World Health Organization (**WHO**) in 1992⁽³⁾ is as follows:

1. Epithelial Cysts:

A. Odontogenic cysts:

- a) Developmental:
 - 1. Odontogenic keratocyst
 - 2. Dentigerous cyst

- 3. Eruption cyst
- 4. Lateral periodontal cyst.
- 5. Gingival cyst
- 6. Glandular odontogenic cyst
- b) <u>Inflammatory</u>:
 - 1. Radicular cyst
 - a. Apical
 - b. Lateral
 - c. Residual
 - 2. Paradental cyst
- **B.** Non odontogenic cysts:
 - a) Nasopalatine duct cyst
 - b) Nasolabial cyst
 - c) Globulomaxillary cyst
 - d) Median cysts

2. Non epithelialized primary bone cysts:

- a. Solitary bone cyst (simple, traumatic)
- b. Aneurysmal bone cyst
- c. Stafnes idiopathic bone cavity

Cysts of the soft tissues:

- 1. Epidermoid cyst
- 2. Dermoid cyst
- 3. Bronchial cyst (lymphoepithelial)
- 4. Thyroalossal duct cyst

MATERIALS AND METHODS:

A total number of 418 biopsies were collected from the department of oral and maxillofacial

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pathology, Collage of Dentistry, Baghdad University through a period of twenty years ranging from 1991-2010. The following data was recorded for alldiagnosed cystic lesions from their files: age, sex, site of lesion and final diagnosis. Cysts involving the salivary glandswere not included in this study.

RESULTS

The total number of biopsies studied was 5356, of which 418 werediagnosed as cystic lesions comprising 7.08%. Out of 418 cysts, 348 were diagnosed as odontogenic cysts that comprised 83.25%, while nonodontogenic cysts were 14 cases that comprised 3.34% of the totalnumber. Distribution of these cysts is shown in table 1.

There were 50 cystic lesions comprising 14.37% of the total amount, which contained odontogenic epithelium but without any other clear diagnostic features that leads to a definite histopathological diagnosis. These lesions were grouped under the heading of odontogenic cysts that lacked any characteristic histopathological features that enabling definit diagnosis. One non-epithelial cystic lesion was recorded.

Periapical cyst was the most common cystic lesion (155 cases) forming 39.39% of the total number of cysts registered, with most cases occurring in the maxilla (23.3%). Next come odontogenic keratocyst (19.25%), followed by dentigerous cyst (14.94%) and the least predominant odontogenic cyst was calcifying epithelial odontogenic cyst (1.15%).

There were 14 cases of nonodontogenic cysts, of which epidermoid cyst was the most predominant cyst (0.96%), followed by globulomaxillary and nasopalatine cysts (0.72% for each), aneurysmal bone cyst (0.48%) and the least nonodontogenic cysts were dermoid and branchial cysts (0.24% for each). (Table 1). Concerning the site distribution, most cystic lesions occurred in the maxilla (57.2%), followed by the mandible (36.3%), soft tissue (4.8%), and the least number was the maxillary sinus (1.7) as it was showed in table 2. Concerning the age distribution of the cases, the highest incidence of cystic lesions occurred at the age of 21-30 (31.10%) with male predominancy as it was showed in table 3.

DISCUSSION

Periapical cyst has been reported as being the most common cystic lesion of the jaw. It was reported that 55% of all jaw cysts studied were radicular and residual cysts.⁽⁵⁾ Others reported a higher incidence (65-75%),⁽³⁾ this is because residual cysts were

grouped together with periapicalcyst due to the fact that the residual cyst is a periapical cyst remaining in the jaw bone after extraction of the causative tooth.⁽⁴⁾

In this study, we reached the same conclusion that the inflammatory odontogenic cysts are the most common cystic lesion reported in the jaws bones of Iraq's population (39.39%). Odontogenic cysts as a whole comprise about 90% of all jaw cysts.⁽³⁾ In this study, they formed 83.25%. The main site of occurrence of odontogenic cysts was in the maxilla (57.2%).

Odontogenic keratocyst had the highest incidence of developmental odontogenic cysts (19.25%). Most studies show varied incidence of this cyst among odontogenic cysts. Some report (10-12%).⁽⁴⁾ Others reports (5-10%).⁽⁶⁾ This variation could be due to variation in sample size or the way of collection of the samples.

In most studies, dentigerous cyst was reported as the most frequent developmental odontogenic cyst. Benn and Aitini (1996)⁽⁷⁾ reported that 16.6% of all jaw cysts were dentigerous cysts. Soames and Southam (1999) reported an incidence of 10-15%. Cawson (2002) reported a frequency of 15-18%. In this study, dentigerous cyst formed 14.94% of all cysts which is in agreement with the studies mentioned above, but dose not rank as the most frequent odontogenic cyst that may be send for routine histopathological diagnosis. This lesion develops from fluid accumulation between reduced enamel epithelium and the enamel surface of an impacted tooth. Many maxillofacial surgeons may base their diagnosis on both radiographical and gross appearance of the lesion at the time of surgical operation. Also the decreased incidence of dentigerous cysts in this study as compared toother studies may be due to the decreased incidence of impacted teeth as opposed to western studies.

The inflammatory type of odontogenic cysts showed a predilection for the maxilla. This has also been reported as being 60%.⁽³⁾ This high incidence is thought to be due to the large number of periapical cysts which arise as a result of trauma to the anterior teeth in addition to caries, whereas in the lower jaw this factor is generally absent, leaving caries as the principal cause of periapical cysts.

Cystic lesions are generally seen in young adults especially the odontogenic developmental lesions.^(8,9) This was also reported in our study where the highest incidence was in the age group 21-30. Male were affected more than female. Age and sex distribution were in accordance with various studies in which cystic lesions were generally seen in young males.

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In general, the findings of this study were in agreement with other reports, the difference being that the odontogenic keratocyst had a higher inci-*(Table 1) Distribution of cysts according to types*

dence as compared to dentigerous cyst while most other studies reported the dentigerous cyst as being the most common odontogenic developmental cyst.

Type of cyst	Number	%
Keratocyst	67	19.25%
Dentigerious cyst	52	14.94%
Odontogenic cyst	50	14.37%
Calcifying epithelialodontogenic cyst	4	1.15%
Periapical cyst	155	39.39%
Residual cyst	20	5.74%
Nasopalatine cyst	3	0.72%
Globulomaxillary cyst	3	0.72%
Epidermoid cyst	4	0.96%
Dermoid cyst	1	0.24%
Branchial cyst	1	0.24%
Aneurysmal bone cyst	2	0.48%
Total	362	100%

(Table 2) Site distribution of cysts

Site	No.	%
Maxilla	239	57.2%
Mandible	152	36.3%
Maxillary sinus	7	1.7%
Soft tissue	20	4.8%
Total	418	100%

(Table 3) Age distribution of cysts

Age group	Number	%	Male	Female	
0-10	25	5.98	16	9	
11-20	101	24.16	45	56	
21-30	130	31.10	78	52	
31-40	61	14.59	31	30	
41-50	42	10.04	26	16	
51-60	28	6.69	12	16	
61-70	25	5.98	18	7	
71-80	4	0.95	4	0	
80+	2	0.47	2	0	
Total	418	100%	232	186	

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