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Decontamination of Dental Implants Using Two Different Solutions and Different Contact Times: An Experimental Study

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ABSTRACT

Background: Osseointegration used in relation to titanium metallic implants, the idea now applies to all biomaterials that can osseointegrate in facial bone, such as ceramics which used as bone substitutes. **Objective:** to compare the decontamination of dental implants using chlorhexidin and ozonated water contact time (1 & 3 min.) each prior to implantation in rabbits' tibial bone. **Patients and methods:** Twenty eight healthy New Zealand white rabbits weighing 2.5-3 kg were used for this study and divided equally into two groups, then each group subdivided into two subgroup each of seven. Dental implants decontaminated by chlorhexidine 2% and ozonated water. All animals have been sacrificed 8 weeks post implantation. The operated tibia have been harvested and preserved in formalin 10%. New bone and the osteoid matrix content around the dental implants have been evaluated histologically. **Results:** All animals of the experiment stayed a life till the end of the experiment with no complications what so ever except some members in groups A1, B1 and B2 shows sinus tracts oozing pus at the surgical site. The acute abcess (A.A) in rabbit tibial bone showed a significant decrease in groups A2, B1 and B2 in comparison with group A1. The lowest decrease was observed in groups A2 and B1.The subacute abcess (SA.A) showed a significant increase in rabbit bone of group B1 when compared with group A1. The non-abcess (N.A) revealed a significant increase in rabbit bones of groups A2 and B2 comparatively with those of group A1, however N.O.M in rabbit bone of group B1 showed insignificant statistical change when matched with group A1. **Conclusion:** Decontamination of already contaminated dental implant with human saliva prior to implantation using chlorohxidine 2% for three minutes give decent and proper result on the surrounding bony tissues than using ozonated water from ozone generator device (output 400 mg/hour) when used for the same exact time.

Keywords: Dental Implants; Decontamination ; Ozone ; Chlorhexidine

Introduction

Osseointegration was originally defined as a direct structural and functional connection between ordered living bone and the surface of a load-carrying implant. Nowadays it said that an implant is said to be osseointegrated when there is no progressive relative movement between the implant and the bone with which it is in direct contact. Although the term

osseointegration was initially used with reference to titanium metallic implants, the concept currently applies to all the biomaterials that can be osseointegrate in bone of the face as ceramics used as bone substitutes **(1)**.

The mechanism of osseointegration is related to many factors, including the quality of the bone , bone type , the biocompatibility and surface characteristics of the implant material, the surgical technique, healing factors including age, oral hygiene, systemic disease, aseptic germ free surgical environment and functional loading **(2)**.

Osteomyelitis related to dental implants is a rare and serious complication. A prolonged antibiotic at high dosage together with aggressive surgical treatment is needed for its containment. Increasing the knowledge among dental surgeons and achieving correct surgical techniques are essential in order to reduce the risk of osteomyelitis Therefor, aseptic germ free of both surgical environment and implant body has great deal in the success of the implantation and the ossiointegration process and lower the risk factors of such conditions **(3)**.

Chlorhexidine, also known as chlorhexidine gluconate (CHG) is non-corrosive disinfectant and antiseptic that is used for skin disinfection prior to surgery and to sterilize surgical instruments. It may be used both to disinfect the skin of the patient and the hands of the surgeons. It's acting by disrupting the cell membrane. because the positively charged chlorhexidine molecule interact with negatively charged groups on the bacterial cell wall there for permits the agent to go within the cytoplasm and kill the microorganism **(4)**.

Ozone (O3) is a triatomic molecule, consisting of three oxygen atoms. Its molecular weight is 47,98 g/mol. Ozone is thermodynamically highly instable compound that according to system conditions like temperature and pressure, it decomposes to pure oxygen with a short half-life. There are several known actions of ozone on human body, such as immunostimulating, analgesic, antihypoxic, detoxicating, broad-spectrum antimicrobial, bioenergetic and biosynthetic by increasing the metabolism of carbohydrates, proteins and lipids **(5)**.

Rabbits are very excellent choice experimental studies as Rabbit it's a member of the Lagomorpha order, is the closest phylogenetic relative to humans, after the primates. It possesses greater acceptability as a laboratory mammal than primates in terms of husbandry, breeding ease, distinct small periods of generations, cost effectiveness and legal ethical conveniences **(6)**.

So by using different solution and different exposure time to achieve disinfection of Dental Implants we could know the better way of doing so and how that will affect the surrounding bony structure. This study aimed to compare the decontamination of dental implants using two different solutions and different contact times and their effect on the surrounding bonny structure. The solutions were used for decontamination of dental implants are chlorhexidin 2% and ozonated water with contact time one and three minutes each prior to implantation in rabbits' tibial bone.

Materials and Methods

Twenty eight healthy New Zealand white rabbits weighing 2.5-3 kg, and the animals were housed in the animal house at the faculty of veterinary medicine, Suez Canal University. Heads of tibia of the animals for the implantation of samples. Rabbits' tibiaehas been widely used as an animal model to study and testing dental implants **(7)**.

Materials:

1-Minidental implant (MDI): is self-tapping screw type dental implants 28 in number each is 8mm long and 3.5mm in diameter (Nucleoss with surface treatment by acid etch with SLA, Turkey).
2-Ozon generator device: wy-0266 (ozone output 400mg/hour), Wanyang, China.
3-Chlorohexidine 2%: with surface modifier **(**Adam dent**,** Egypt**)**.

Sample size calculation:

The sample size for this study was calculated according to Jaykaran and Tamoghna (2013) used the following equation: $\mathbf{N = \frac{(Z_\alpha)^2 * (SD)^2}{(d)^2}}$

N = Total sample size, SD = Standard diversion of variable, d = Absolute error or precision**,** Z_α= Is Standard normal variation and its equal 1.96 at P< 0.05

Experimental design:

Twenty eight rabbits used for this study and divided equally into two groups, then each group subdivided into two subgroup each of seven, as follow:

GROUPS	DESCRIPTIVE	no. of samples
	Subgroups	
Group A	Subgroup (A_1)	7
	Subgroup (A_2)	7
Group B	Subgroup (B_1)	7
	Subgroup (B_2)	7
Total Samples		28

- **Group A:** Fourteen rabbits used for dental implants decontaminated with chlorhexidine 2% subdivided into:

I- Subgroup (A1) consist of seven rabbits has been used for dental implants decontaminated with chlorhexidine 2% for one minute.
II - subgroup A2 consist of seven rabbits used for dental implants decontaminated with chlorhexidine 2% for three minutes.

- **Group B:** Fourteen rabbits used for dental implants decontaminated with ozonated water further subdivided into:

I - subgroup (B1): consist of seven rabbits have been used for dental implants decontaminated with ozonated water for one minute.
II - subgroup (B2): consist of seven rabbits have been used for dental implants decontaminated with ozonated water for Three minutes.

Surgical procedure

The procedures were approved by the institutional animals' ethics review board of Suez Canal University. Animals were anesthetized by an IM injection of ketamine hydrochloride-xylazinemixture at 35 and 5 mg/kg. Animals were shaved for twice the size of the expected surgical field with an electric razor. The surgical area was cleaned with gauze and 2% chlorhexidine solution.The animal was draped and fixed with clamps on a sterile, impermeable covering to isolate the disinfected area.

Surgical protocol for MDIs; 3-5 cm longitudinal skin incision just distal to the tibia femur joint was made. The tibial head was exposed subperiosteally and an osteotomy performed with placed pilot drill over the entry point and lightly pumped up and down under copius saline irrigation just to enter the cortical bone for the MDIs. This was used for initial bone drilling to depth of 0.5 mm then serial drill used. The MDI (3.5 mm×8 mm) vial was opened and the body of the implant was firmly grasped with a sterilized locking pliers. We contaminate it with human saliva then with mentioned solution and time for each group. The titanium finger driver was attached to the head of the implant. The implant was transferred to the site and rotated clockwise while exerting downwards pressure. This began the self-tapping process and until noticeable bony resistance encountered when it touched the lower cortical plate. The winged thumb wrench was used for driving the implant deeper into the bone.

Length of the procedure was approximately 1 h. Following placement of the implants, the wound was sutured in layers. The underlying muscle, fascia, and dermal layers were sutured with the help of Vicryl (Polyglactin 910) suture with 3/8 circle reverse cutting needle. The skin was sutured to a primary closer with the same suture material. All the animals received one MDI in the head of the tibia. Therefore, total 28 mini dental implants were inserted.

Post-surgical treatment

After the surgical procedure, the animals were housed in a cage under the supervision of a veterinary doctor until they came out anesthesia. The rabbit was observed every 2hr. to check the wound for infection. The wound was protected with povidone iodine ointment. The rabbits were allowed immediate weight bearing as tolerated; therefore, they had no restraints on weight bearing. The rabbit was given a dose of Cephalexin 12 mg/kg 0.5 ml I.V. once IP and Carprofen 2mg/kg,S.C./8hr/3days. The wound was cleaned with betadine. After 6 weeks each group has been examined to find out any surgical defects.

Euthanasia

The animals were euthanized at 6 weeks respective by an overdose of thiopental sodium IV.

Specimen retrieval

The implants along with their surrounding bone were excised with a surgical saw right away following the euthanasia. The excess tissue was dissected and the specimens were collected per each group to take plain x-ray to ensure the presence of the implant and then the specimens were removed in block with a margin of surrounding bone of about 5–10 mm, immediately put into the 10% formaldehyde solution.

Histological Preparation:

In order to achieve optimal results when processing calcified tissues, it is important to determine the point at which decalcification is complete. The formalin preserved bone tissues were processed in an automated tissue processor. The processing consisted of an initial two step fixation and dehydration. Fixation comprising tissue immersion in 10% buffered

formalin for 48 hours, followed by removal of fixative in distilled water for 30 minutes. Dehydration was then carried out by running the tissues through a graded series of alcohol (70% -100%). The tissue was initially exposed to 70% alcohol for 120 minutes followed by 90% alcohol for 90 minutes and then two cycles of absolute alcohol, each for one hour. Dehydration was then followed by clearing the samples in several changes of Xylene. It consisted of tissue immersion for an hour in a mixture comprising 50% alcohol and 50% Xylen. Followed by pure Xylene for one and a half hour. Samples were then impregnated with molten paraffin wax, then embedded and blocked out. Paraffin sections (4–5 μm) were stained with hematoxylin and eosin. Stained sections were examined for presence of urolithiasis and the accompanying tissue pathological changes **(8)**.

RESULTS

The present study showed group A1 implants that went under decontamination by chlorohexidine 2% for one minute show 42.86% of acut supurative abcess(a.a)and 42.86% of subacute suppurative abcess(s.a.a) with percentage of 0% reminiscing abcess(r.a)and percentage of 14.28% no abcess (n.a)as well as surgical wound healing with marked evidence of abcess or ulcers in 6 of 7 rabbits. Group A2 implants that went under decontamination by chlorohexidine 2% for three minutes show no cases of acut supurative abcess(a.a)or subacute suppurative abcess(sa.a))with percentage of 57.14% reminiscing abcess(r.a)and percentage of 42.85% non abcess(n.a)as well as good surgical wound healing with no evidence of abcess or ulcers.

Group B1 implants that went under decontamination by ozonized water for one minute show 0% of acut supurative abcess(a.a)and 57.14% subacute suppurative abcess(sa.a))with percentage of 28.57% reminiscing abcess(r.a)and percentage of 14.28%% non abcess(n.a)as well as surgical wound healing with marked evidence of abcess or ulcers in 6 of 7 rabbits. GroupB2 implants that went under decontamination by ozonized water for three minutes show 28.57% of acut supurative abcess(a.a)and no cases of subacute suppurative abcess(sa.a))with percentage of 28.57% reminiscing abcess(r.a)and percentage of 42.85% non abcess(n.a)as well as surgical wound healing with marked evidence of abcess or ulcers in 4 of 7 rabbits. The types and percentages of abcess in different experimental groups are calculated and illustrated in the following statistics chart **(Fig. 1)**.

Examined sections from rabbit tibial bone at the platinum implants- bone surface interface of (Group A1) pointed out and declared many histo-morphologic characteristic changes which were represented by high percentage of reactive acute and subacute suppurative abcess (6 out of 7 cases were positive for abcess) . The pronounced micro-morphologic changes were characterized by osteo-necrotic, osteolytic and osteomalacic multi-focal areas surrounded by dense infiltration of polymorph-leucocytes , lymphocytes and histiocytes, sometimes with osteoclastic reactivates. The surrounding bone marrow showed marked reactive changes with remarkable sinusoidal dilatation, Polymrph-leucocytic active proliferation and occasional monocytic and plasmactocytic aggregations. No- osteomyitis case showed apparently healthy bone surface interface with normal osteoblastic rimming and surrounding physiologically active bone marrow .Occasional sinusoidal dilatation and focal monocytes and plasma cells aggregates were recorded **(Fig. 2)**.

Examined sections from rabbit tibial bone at the platinum implants- bone surface interface of (Group. A2) revealed reminiscing changes of previous abcess in (4 out of 7 cases) represented by fibrino-inflammatory and osteonecrotic changes with gradual reparative processing as manifested by phagocytic removal of the necrotized bony tissue, decreased intensities of the vascular and cellular hemodynamic and exudative inflammatory reactions

respectively with occasional predominance of lympho-plasmacytic proliferative changes in the adjacent bone marrow . The remaining cases (3 out of 7 cases) showed apparently healthy compact and trabecular bone and physiologically active bone marrow with a more reactive lympho-plasmacytic proliferative changes , mostly as a consequent post-inflammatory process **(Fig. 3)**.

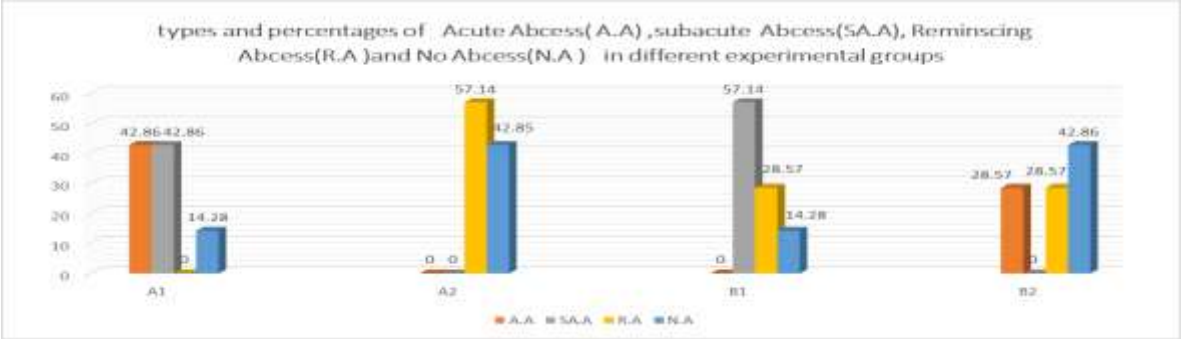

Fig. (1) : Showing The types and percentages of abcess in different experimental groups

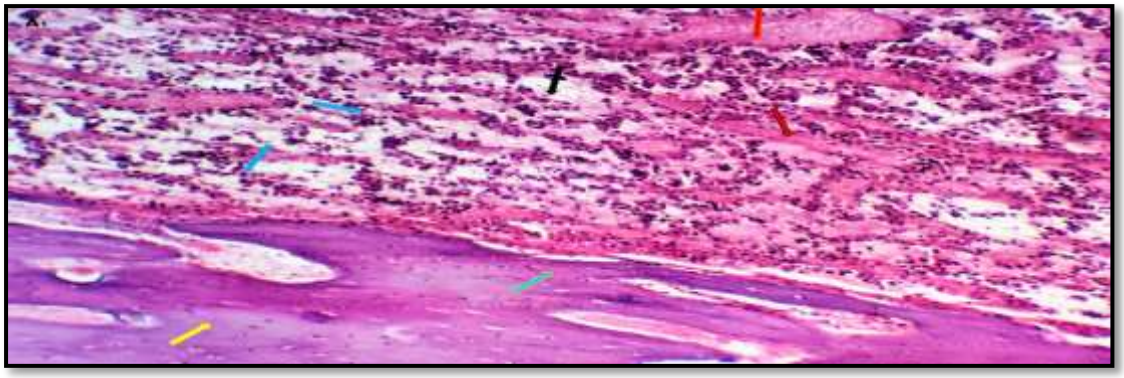

Fig. (2): Photo-micrographs from rabbit tibial bone at the platinum implants-bone surface interface of (Group. A1). Acute and subacute suppurative abcess characterize by osteo-necrotic, osteolytic and osteomalacic multi-focal areas (green arrows) surrounded by leucocyte infiltration osteoclastic reactivates (blue arrows). The surrounding bone marrow shows marked reactive changes with remarkable sinusoidal dilatation, Polymrph-leucocytic active proliferation and occasional monocytic and plasmactocytic aggregations (red & blue arrows). Interstitial edema can be seen (black arrows) Non-osteomyitis case showed apparently healthy bone surface interface with normal osteoblastic rimming and surrounding physiologically active bone marrow (yellow, blue,red arrows). Sinusoidal dilatation and focal monocytes and plasma cells aggregates are seen (red & blue arrows) (H&E X 200).

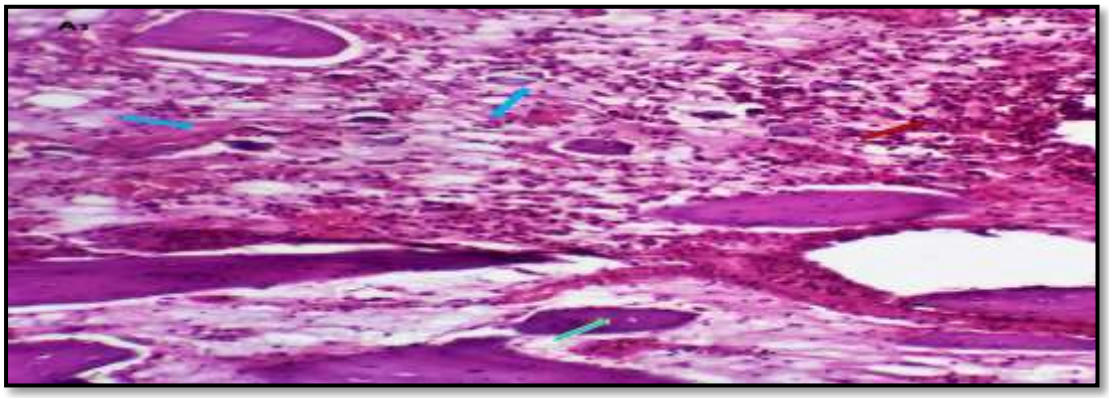

Fig. (3): Photo-micrographs from rabbit tibial bone at the platinum implants-bone surface interface of (Group. A2) reminiscing changes of previous abcess represents by fibrino-inflammatory and osteonecrotic changes (blue, red & green arrows) with gradual reparative processing as manifested

by phagocytic removal of the necrotized bony tissue, decrease intensities of the vascular and cellular hemodynamic and exudative inflammatory reactions respectively with occasional predominance of lympho-plasmacytic proliferative changes and a few multi-nucleated giant cells in the adjacent bone marrow. Apparently healthy compact and trabecular bone and physiologically active bone marrow with a more reactive lympho-plasmacytic proliferative changes (blue arrows) (H&E X 200).

Examined sections from rabbit tibial bone at the platinum implants- bone surface interface of (Group. B1) demonstrated micro-morphologic changes of a relative mild to moderate abcess changes, reminiscing changes of abcess and a normal osteohistologic characters with a range values of (4:2:1) .A subacute suppurative abcess (4 out of 7 cases) were represented by mild to moderate osteodegenerative and osteonecrotic bony tissues , sometimes partially fragmented or malacic and surrounded by mild to moderate numbers of plasma cells ,polymorph leukocytes and lymphocytes. The reminiscing abcess (2 out of 7 cases) was characterized by mild focal osteodegenerative changes with a surrounding moderately active bone marrow entangling variable numbers of plasma cells , monocytes and a few polymorphonuclear cells . A normally looking bone tissue with a reactive bone marrow and a focal compensatory osteo-chondrogenesis were seen in (1 out of 7 cases) **(Fig. 4)**.

Examined sections from rabbit tibial bone at the platinum implants- bone surface interface of (Group. B2) revealed acute suppurative abcess, reminiscing abcess changes and apparently normal bone structures enclosing a moderately reactive bone marrow with a range values of (2:2:3). Acute abcess (2 out of 7 cases) was represented by exudative sero-fibrinous -inflammatory , osteonecrotic and focal osteomalacic changes, marked sinusoidal dilatation and inflammatory cells aggregations with a predominance of the polymorphonuclear leucocytes. A few lymphocytes and plasma cells were also participated. The reminiscing abcess (2 out of 7 cases) characterized by mild focal osteodegenerative changes with a surrounding moderately active bone marrow entangling variable numbers of monocytes and a few polymorphonuclear cells. The remaining cases (3 out of 7) showed apparently healthy compact and trabecular bone and physiologically active bone marrow with more reactive lympho-plasmacytic proliferative changes **(Fig. 5)**.

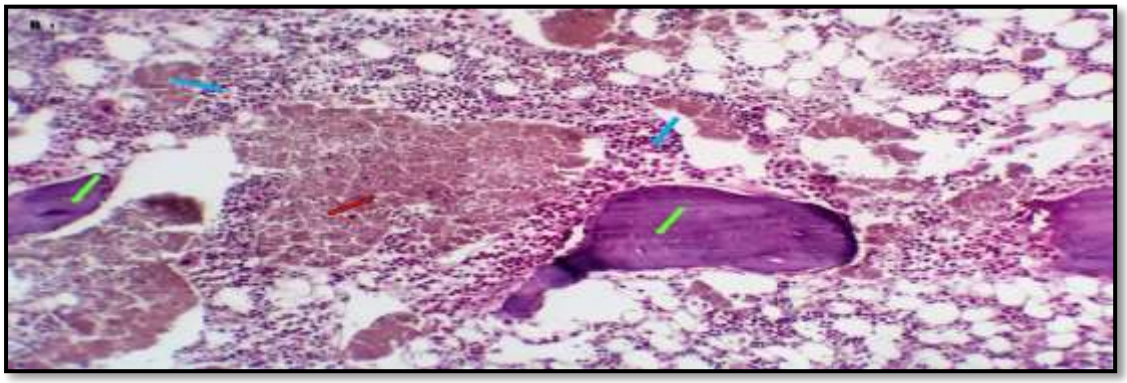

Fig. (4): Photo-micrographs from rabbit tibial bone at the platinum implants- bone surface interface of (Group. B 1). A relative mild to moderate abcess changes, reminiscing changes of abcess and a normal osteohistologic characters. A subacute suppurative abcess is represent by mild to moderate osteodegenerative and osteonecrotic bony tissues , sometimes partially fragmented or malacic and surrounded by mild to moderate numbers of plasma cells ,polymorph leukocytes and lymphocytes (green, blue and red arrows) The reminiscing abcess is characterize by mild focal osteodegenerative changes with a surrounding moderately active bone marrow entangling variable numbers of plasma cells, monocytes and a few polymorphonuclear cells (geen , blue & red arrows). A normally looking bone tissue with a reactive bone marrow and a focal compensatory osteo-chondrogenesis is seen (yellow arrows) H&E X 100.

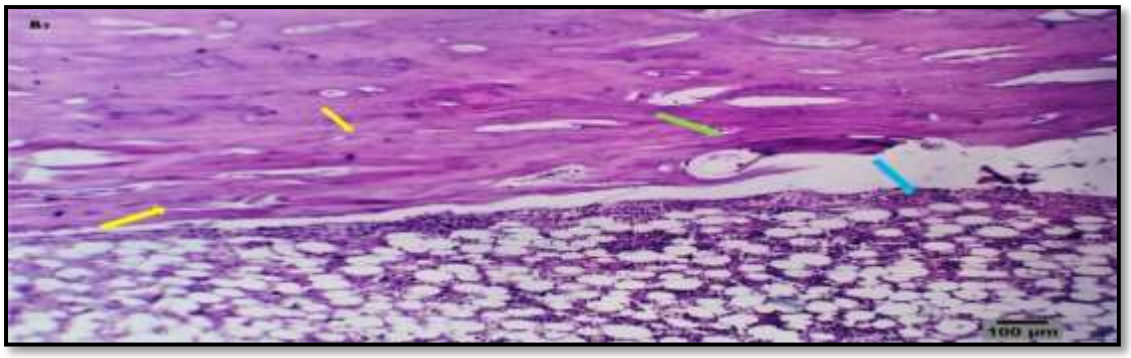

Fig. (5): Photo-micrographs from rabbit tibial bone at the platinum implants- bone surface interface of (Group. B 2). Acute suppurative abcess, reminiscing abcess changes and apparently normal bone structures enclosing a moderately reactive bone marrow . Acute abcess is represents by exudative sero-fibrinous-inflammator, osteonecrotic and focal osteomalacic changes(green &yellow arrows), marked sinusoidal dilatation and inflammatory cells aggregations with a predominance of the polymorphonuclear leucocytes (blue and red arrows). A few lymphocytes and plasma cells are also participated . The reminiscing abcess is characterize by mild focal osteodegenerative changes(yellow arrows) with a surrounding moderately active bone marrow entangling variable numbers of plasma cells , monocytes and a few polymorphonuclear cells(green , black & blue arrows). The remaining cases shows apparently healthy compact and trabecular bone and physiologically active bone marrow with a more reactive lympho-plasmacytic proliferative changes(yellow , green & blue arrows) (Scale bars 100 um).

DISCUSSION:

Dental implants for its relatively long life than many other animals besides having the ability for withstanding many different surgical procedures as well as large amount of bony areas that we can perform implantation on with many numbers of implants. Sheep is proven to be good models for dental implants and bone grafts experiments for the past exact reasons **(9)**.

Rabbits are very excellent choice in experimental studies as rabbit it's a member of the Lagomorpha order, is the closest phylogenetic relative to humans, next to primates. It possesses greater acceptability as a laboratory mammal than primates in terms of husbandry, breeding ease, distinct short periods of generations, cost effectiveness, and legal ethical conveniences **(10)**.

For the same reason that we separated the total number of 28 rabbits into two groups in order to carry out the comparison, we used rabbits as the experimental models in this study.

Many researcher used to perform mini dental implant in rabbits mandible sites for implants that were far enough from the apices of the teeth in the mandibular molar area were chosen **(11,12)**. But other studies prefer other sites for conducting dental implant related experiments. Rabbits' tibiae has been widely used as an animal model by various researchers to study and testing dental implants **(13)**. As in the current study we adopted this approach.

we used rabbits' right tibias as the site of choice for conducting the experiment as Twenty eight male New Zealand rabbits aged 8- 10 weeks and weighting 2.5–3.0 kg in animal house of faculty of veterinary medicine of suez canal university has been used, ketamine and xylotazine HCL used intramuscularly for anesthesia of the animals(ketamine 35 mg/kg , xylotazine Hcl 5mg/kg IM). Sites for implants was at the tibial bone **(14)**.

Saliva has great role in the healing of intraoral wounds because of having many factors that help and promote wound healing in addition of its analgesic and hemostasis roles. it contain antimicrobial effect and many different growth factors, several proteins and peptides that protect the oral cavity against microbial infections and promote wound healing **(15)**.

While per implant osteomyelitis is a common complication for denture wearers, as a number of issues relating to dental implants and salivary contamination. Also, through oral analysis using rinses, swabs, and aspirates. One of the primary caustic species of osteomylitis, S. aureus, may be present in the lips of elderly individuals more frequently than previously considered. Frequency of isolation of *Staphylococcus spp* may be age related and it is regularly recovered from denture plaque. *S. aureus* colonises denture surfaces and is not eliminated with standard denture cleansers. As Rinsing with chlorhexidine has been found to decrease the complications during the implant submerged period, however, *S. aureus* may have a particular adherent to titanium surfaces. Also Immunological factors may also be involved **(16)**. In the present study we investiged the role of saliva in causing periimplantitis and periimplant bone infection despite of going through series of decontaminations methods to avoid so.

Saliva contamination during dental implant implantation can compromise implant-bone contact in augmented areas, but it has no discernible impact on newly formed bone farther away from the implant surface, according to study results on contaminated implants in augmented bone **(17)**. There far salivary contamination should be avoided during placement of dental implants in augmented areas. **Jinno et al. (17)** in agreement with the current study has proven saliva has marked role in bone of which we performed the osteotomy to develop infection and osteomyelitis in such immunocompromised species as rabbits.

None of the methods described in the literature can completely and perfectly decontaminate against oral microorganisms or prevent the risk of infectious complications dispite his widely uses of chlorohexidine in his study but the current study used higher percentage of chlorohexidine despite the bony area of question has to go through such invasive procedure as drilling osteotomy hole for implantation in delicate species as rabbits. **(18)**.

Osteomyelitis associated with implant spreads mainly by local extension rather than by hematogenous route. The literature reviewed and the results of the present study offer important information regarding the demographics of osteomyelitis associated with implant placement. These findings are in agreement with studies reporting osteomyelitis may be initiated by faulty technique or septic implantation and for less extend the patient health **(19)**.

In our study, osteomyelitis has been shown as ulcers and sinus tract through the surgical site in many members of groups A1, B1 and B2. This finding in agree with **Mandell, et al. (20)** who concluded that osteomyelitis in long bone eventually may induce the formation of a sinus tract which may result if the cloaca communicates with the skin surface through the soft tissues and of course skin ulcers.

Previous studies has been conducting on dental implants as it come to the histological part to perform the ground undecalcified sections to measures the bone implants contact and degree of osseointegration **(12,21)**.

We performed decalcified section to know the kind of cells presented and type of the bony tissue and presence of any inflammation and it's degree beside the efficacy of decontamination solutions of each group and it's tolerance by the rabbit's bony tissues. This finding was in agreement with **Rajendiran et al. (22)** who preferred decalcified sectioning to laboratory examins bone inflamation, infections and osteomyelitis

According to **Abraham et al. (23)** who revealed that chlorohexidine as antiseptic can be used at each stage of implant treatment for pre-surgical mouth wash for reduction of bacterial load, Intra /extraoral scrub before implant surgery- as surface antiseptic, Hand scrub before implant surgery- as surface antiseptic, Post-surgical rinse till closure of incision line

and Control of subsequent infections As it kills high percentage of bacterial community in just few seconds.

Simillary, **Fiorillo (24)** reported that chlorhexidine is used in many areas of dentistry. It is used widely for as a cure for cases of mucositis, use of chlorhexidine gel inside the fixture/abutment of a dental implant connection can decrease the peri-implant peri-implantitis, for the prevention of alveolitis or for conservative or endodontic treatment. So, the positive effects of chlorhexidine are much presented.

In a study of **Sajjan et al. (25)** showed that chlorohexidine as antiplaque agent, as a root canal irrigant, prevention of caries by suppression of S. mutans, prevention of sceondary infection in apthous ulcers and in alveolar osteitis. They showed a promisng results as an antifungal agent ascertained by its role in the management of denture stomatatis and implant associated biofilms.

Moreover, **Azarpazhooh&Limeback (26)** found a good evidence of ozone biocompatibility with human oral epithelial cells, gingival fibroblast, and periodontal cells but there is questionable evidence of antimicrobial efficacy of ozone but some evidence that ozone is effective in removing the microorganisms from dental unit water lines, the oral cavity, and dentures. And totally insufficient evidence for the application of ozone in oral surgery and implantology. Despite the promising in vitro evidence, the clinical application of ozone in did not achieve a strong level of efficacy and cost-effectiveness.

However, **Mirmortazavi (27)** revealed that Ozone (O3) is recognized as a strong oxidative antimicrobial agent. Ozonated water (OW) has been considered as an effective disinfectant agent against oral pathogens, including *Candida* and so far Ozone is a versatile substance in dentistry, caries control, root canal disinfection, avulsed teeth disinfection before re-implantation, surgery as a hemostatic agent, postsurgical wound healing, sterilization of implant and bone surface, and tissue regeneration stimulation at implantation site. This finding are in agreement with the current study**.**

Difficulty in handling the instrument in ozonated water for such purpose as it has all of the following disadvantages including stormy and filled with bubbles, ozonated water has high dissolving power for rubber latex of the operator's gloves, complicated in use, the sharp smell of the ozone gas irritates the operator, short shelf life, need special equipment, and electricity dependent.

CONCLUSION:

Decontamination of already contaminated dental implant with human saliva prior to implantation using chlorohxidine 2% for three minutes give decent and proper result on the surrounding bony tissues than using ozonated water from ozone generator device (output400 mg/hour) when used for the same exact time in addition to chlorohexidine having advantages of being less expensive, available, good validity period, decent shelf life, more easy to handle , and no need for special devices or electricity**.**

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Author contribution: Authors contributed equally in the study.

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