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Medhat A. El-Zainy

Ahmed Mahmoud Halawa

Radwa Taher Elsharkawy
radwaelsharkawy11@gmail.com

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Effect of the parotid salivary gland on calcium and amylase enzyme levels in blood and its influence on bone healing in albino rats (Histological and radiographic study)

Medhat A. El-Zainy Prof Dr [a], Ahmed Mahmoud Halawa Assistant Prof Dr [a], Radwa Taher Elsharkawy Lecturer [b, *]

[a] Oral Biology, Faculty of Dentistry, Ain Shams University, Egypt
[b] Oral Biology, Faculty of Oral & Dental Medicine, Future University, Egypt

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ABSTRACT

The healing potential of bone is influenced by a variety of biochemical, cellular, hormonal and pathological mechanisms. As previous studies stated that parotid salivary glands may have endocrinal role, the aim of this study was to evaluate the effect of parotidectomy on bone healing and on calcium and amylase enzyme levels in blood. The rats were divided into two groups; control and experimental group. The control group was subjected to unilateral surgical mandibular defects, while the experimental group was subjected to the same procedure in addition to bilateral surgical removal of the parotid glands. Each of the control and experimental groups was further subdivided into 3 subgroups, **A, B** and **C** according to the time of termination corresponding to 4, 8 and 12 weeks respectively. Blood samples were obtained in order to determine calcium and amylase levels in blood. The surgically defected mandibles of each subgroup were analyzed postoperative to determine the radiographic bone density of the surgical defect throughout the healing process, then processed and examined histologically. Examination of the H & E stained sections of the mandibles at 4 weeks showed minimal bone formation from the defect margin of the experimental group in comparison with the control group. At 8 weeks, the experimental group showed increase of bone formation from the defect margin. At 12 weeks, the center of the defect was filled by a considerable amount of spongy bone and a definite reversal line between new and old bone. The Masson trichrome stained sections of the experimental group at 12 weeks presented a considerable amount of green collagen fibers. The average (mean) percentage of radiographic bone densities of the surgical defect of the experimental group slightly raised to 82.06 at 12 weeks. The serum amylase level at 4 weeks was less than the normal value then was slightly increased at 8 weeks and finally at 12 weeks increased more than the normal value. However, the serum calcium level was within the normal value in all experimental and control subgroups. It was concluded that bilateral parotidectomy in albino rats resulted in delayed bone healing and was associated with an initial drop in serum amylase level at 4 weeks, however serum amylase level was self-compensated at 8 and 12 weeks postoperatively, while it didn't significantly influence serum calcium level.
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1. Introduction

Bone is a specialized mineralized connective tissue which makes up the skeletal system together with cartilage. Bone serves three major functions, a mechanical function serving as a support and site for muscle attachment for locomotion, a protective function for vital organs and bone marrow and finally a metabolic function where it acts as a reserve for calcium and phosphate used for maintenance of serum homeostasis and electrolyte balance [20].

Healing of bone restores the tissue to its original physical and mechanical properties. Healing occurs in three distinct but overlapping stages; the inflammatory stage, the repair stage and the late remodeling stage [12].

* Corresponding author.
E-mail address: radwaelsharkawy11@gmail.com (R.T. Elsharkawy).
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Bone continuously remodels by coordinated cellular mechanisms to adapt its strength to the changing needs of growth and physical exercise. Old, damaged and unneeded bone is removed by resorption, and new bone is subsequently deposited by formation. Diseases affecting either or both of these processes lead to disturbed calcium homeostasis [19].

Calcium balance refers to the state of the calcium body stores, which are primarily in bone and which are largely a function of dietary intake, intestinal absorption, renal excretion and bone remodeling. Bone calcium balance can be positive, neutral and even negative, depending on a number of factors which include growth, aging, acquired and inherited bone disorders [17].

The salivary glands are generally considered as exocrine glands, which secrete their proteins and fluids externally into a lumen or a duct. However many previous studies stated that parotid salivary glands were found capable of endocrine secretion, dispensing their proteins as amylase directly into the blood stream [10,18].

So the present study aimed to evaluate the regulatory function of the parotid salivary glands on serum amylase enzyme and calcium, also whether the parotid gland has an impact on bone healing or not.

2. Materials and methods

The experimental procedures were conducted in compliance with ethical principles for animal research as reviewed and approved by institution guidelines of Ain Shams University ethical committee.

Forty two adult male albino rats (weighing about 200–250 gm each) were used in this study. They were recorded in The Research Center, Faculty of pharmacy, Future University. The animals were housed in wire mesh dated cages (three rats each) and were fed certified pelleted diet and tap water ad-libitum. Temperature and humidity conditions were controlled as possible on housing the animals during the experimental period.

2.1. The rats were equally divided into two main groups (21 rats each)

2.1.1. Group I (control)

Twenty one rats were subjected to unilateral surgical mandibular defects on the right side using 3 mm trephine bur under copious amount of saline irrigation.

2.1.2. Group II (experimental)

Twenty one rats were subjected to unilateral mandibular defect as in **Group I** in addition to bilateral surgical removal of the parotid glands.

- Bilateral removal of the parotid glands was chosen over unilateral removal to avoid any unreliability of the results as the non-removed gland might compensate for the enzyme deficiency.

Each of the control and experimental groups was further subdivided into 3 subgroups, **A, B** and **C** according to the time of termination corresponding to 4, 8 and 12 weeks respectively [16].

2.2. Surgical procedure

2.2.1. Animals anesthetization

- Surgical intervention was performed under general anesthesia using intraperitoneal injection of Xylaine 5–10 mg/kg and Ketamine 20–40 mg/kg [21].
- Post operative care: Prophylactic antibiotic coverage and analgesics were given for 3 days post-operative [4].

2.3. Blood samples collection

Before each scarifying time of both groups blood samples were obtained in order to determine: Calcium blood level and Amylase blood level.

Blood samples were also taken from not operated rats to act as a base line (normal) value for obtained data comparison. Samples were centrifuged to extract the serum.

2.4. Specimens collection

At the end of the experimental period of each sub group, the rats were terminated by an overdose anesthesia. The surgically defected mandibles were excised free and were fixed in 10% buffered formaldehyde for 24 h.

2.5. Radiographic evaluation

Radiographs of the rats' mandibles were taken using a standard x-ray machine. A digital radiograph of the surgical defected mandibles was taken using vista scan digital radiographic system of both the control and experimental groups at 4, 8 and 12 weeks post-operative.

2.5.1. Radiodensitometric analysis

The mandibles of each **subgroup** of the **control and experimental groups** were analyzed post-operative by the Digora software to determine the radiographic bone density of the surgical defect throughout the healing process. Also a radiographic density of normal bone has been taken to compare each result with the normal bone density of each rat mandible to avoid individual variations.

2.6. Histological examination

After complete decalcification, specimens were processed, infiltrated in paraffin wax and embedded in the center of wax blocks. The embedded specimens were cut into 5 microns thick sections. Specimens were sectioned bucco-lingually to show a wider view of the bony defect and were stained by:

2.6.1. Hematoxyline and Eosin (H&E) stain

After fixation, specimens were washed properly under running water, dehydrated by transferring through ascending concentrations of alcohol. The sections were stained by Hematoxyline and Eosin (H&E) stain to be examined by light microscope.

2.6.2. Masson goldner trichrome special stain

It was used to detect areas of new bone formation as green color while old bone areas without new collagen formation appear reddish in color when examined by light microscope [5].

Paraffin sections were fastened to slides with Masson's gelatin.

2.7. Statistical analysis

The resultant data from radiographic examination and blood samples were analyzed statistically. All gathered records were statistically evaluated by Microsoft Office Excel 2007 Statistical Functions to determine the mean (AVERAGE), the standard deviation (STDEV) and p value (T TEST).

3. Histological results

Examination of the H & E stained sections of the mandible of **sub group IA** (control) showed the experimental cavity partially filled with newly formed woven bone budding from the periphery of the surgical defect. An apparent line of demarcation between new and old bone was obviously detected. Resting lines were also observed, straight and gently undulated between successive layers of bone.

The newly formed woven bone exhibited irregular bone trabeculae containing numerous irregularly arranged entrapped large sized osteocytes with intervening wide marrow cavities. Few inflammatory cells and numerous eosinophilic giant cells were detected. Numerous active basophilic stained fibroblasts were obviously seen in both marrow cavities as well as in the connective tissue of the defect cavity. On the other hand, **sub group IIA** showed minimal bone formation from the defect margin. The newly deposited bone trabeculae were apparently reduced in both thickness and extension in comparison with **subgroup IA**. Numerous dilated blood vessels congested with red blood cells (RBCs) and inflammatory cells infiltrations, were encountered in marrow spaces as well as the connective tissue (Figs. 1 and 2).

In **subgroup IIB,** the H & E stained sections of the experimental defects showed increase of the bone formation from the defect margin. The newly formed woven bone exhibited irregular bone trabeculae with wide marrow cavities. The blood vessels appeared normal with no congestion and few inflammatory cells were sometimes encountered. Scattered Osteoblasts lining the bone trabeculae and the marrow cavities were detected. Osteocytes appeared relatively large in size with darkly stained nuclei and surrounded by lacunae of variable size and shape (Fig. 3).

In **subgroup IIC,** the H& E stained sections presented a considerable amount of spongy bone with relatively small marrow spaces filling a considerable part of the bony defect. A definite reversal line between new and old bone was obvious. The most peripheral part of the bony defect presented an almost uniform definite band of lamellar bone with frequent resting lines. The marrow cavities appeared narrow and lined by osteoblasts (Fig. 4).

The **Masson trichrome** stained sections **subgroup IIA** showed few newly formed collagen fibers bundles lining the defect wall, while the rest of the defect was apparently filled with numerous collagen fiber bundles. In **subgroup IIB,** the amount of newly formed collagen fibers increased, moreover, most of the marrow spaces showed new collagen fibers. Finally in **subgroup IIC,** the masson trichrome stained sections presented a considerable amount of green collagen fibers while the old collagen and the new mineralized bone appeared red (Figs. 5–8).

4. Radiographic statistical results

The average (mean) percentage of radiographic bone densities of the surgical defect of the control subgroups was found to be 56.32% of the normal bone at 4 weeks, which increased to 81.79% at 8 weeks and continued to increase in density till reaching 93.10% of that of the normal bone at 12 weeks. The experimental subgroups average (mean) percentage of radiographic bone densities of the surgical defect at 4 weeks was found to be 48.38% of that of the normal bone, increased to 80.27% at 8 weeks and then slightly raised to 82.06% at 12 weeks. By comparing the results of the surgical sites bone densities of the control and experimental groups statistically; the p value was equal to 0.054 at 4 weeks, at 8 weeks was equal to 0.62 and at 12 weeks was equal to 0.05 (Significance level $p \leq 0.05$) (Chart 1).

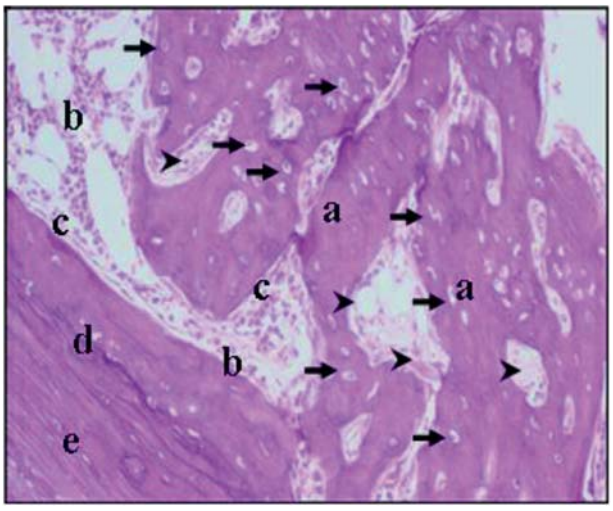

Fig. 1. Photomicrograph of **group IA** showing irregular bone trabeculae budding from the periphery of the surgical defect **(a),** numerous entrapped large sized osteocytes **(arrows)**, wide marrow cavities filled with connective tissue **(b)** few inflammatory cells **(c)**, blood vessels **(arrow heads)**, line of demarcation between new and old bone **(d)** and Resting lines **(e). (H&EX100)**.

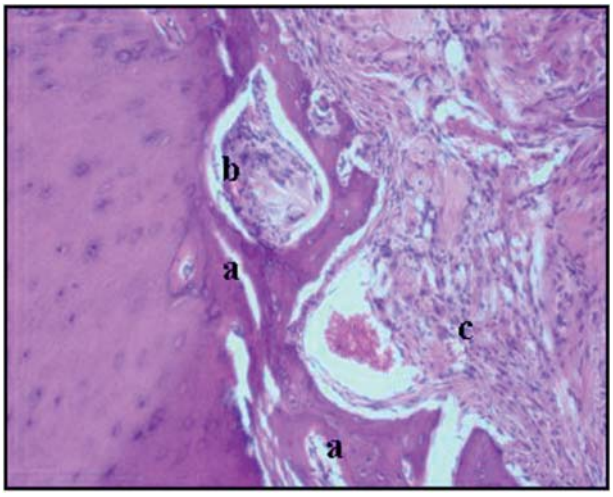

Fig. 2. Photomicrograph of **experimental group A** showing minimal bone formation from the defect margin **(a),** wide marrow cavities **(b)** and inflammatory cell infiltrations in the bone marrow cavities and in the rest of the connective tissues **(c). (H&EX100)**.

5. Hematology statistical results

The results of serum amylase laboratory tests of the control group showed a mean serum amylase level of 640 ± 13.7 U/L at 4 weeks, where at 8 weeks it was 698 ± 24.2 U/L, while at 12 weeks post-operative the mean serum amylase level was 765 ± 28 U/L. While for the serum amylase level for the experimental group the 4 week interval showed a mean of 571.5 ± 30.5 U/L, a mean of 666 ± 20.1 U/L for the 8 weeks interval and a 12 weeks mean of serum amylase of 804.5 ± 13.4 U/L. Statistical evaluation of the results was performed comparing the results of the control and experimental groups at each interval by the p value (with significance mark of p value ≤ 0.05). The calculated p value comparing the results of the control and experimental groups at 4 weeks was 0.173, 8 weeks p value of 0.151 and 12 weeks p value of 0.13.

The results of serum calcium laboratory tests for the control

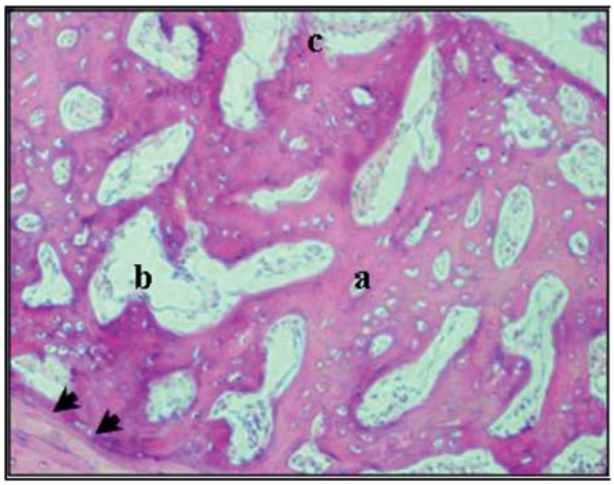

Fig. 3. Photomicrograph of **experimental group B** showing less organized bone trabeculae with large osteocytes **(a)**, wide marrow cavities with few inflammatory cells **(b)**, osteoblastic activities lining the bone trabeculae and the marrow cavities **(c)** and line of demarcation between new and old bone **(arrows)**.

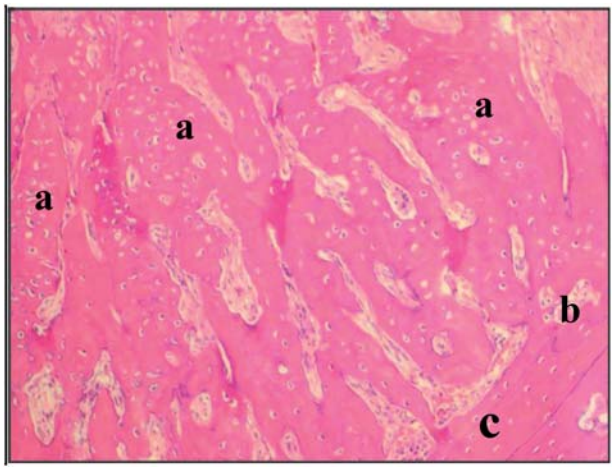

Fig. 4. Photomicrograph of **experimental group C** showing a considerable amount of deposited bone with large osteocytes towards the center **(a)**, a definite reversal line between new and old bone **(b)**, a uniform band of lamellar bone with normal osteocytes and numerous resting lines at the periphery **(c)**. **(H&EX100)**.

group showed a mean serum calcium level of 10.35 ± 0.21 mg/dl at 4 weeks, where at 8 weeks it was 11.2 ± 0.2 mg/dl, while at 12 weeks post-operative the mean serum calcium level of the control group was 11.6 ± 0.21 mg/dl. While for the serum calcium level for the experimental group the 4 week interval showed a mean of 9.9 ± 0.13 mg/dl, a mean of 11.8 ± 0.2 mg/dl for the 8 weeks interval and a 12 weeks mean of serum calcium of 11.9 ± 0.12 mg/dl. The calculated p value comparing the results of the control and experimental groups at 4 weeks was 0.061, 8 weeks p value of 0.014 and 12 weeks p value of 0.142 (Chart 2 and 3).

6. Discussion

Histological results of the present study showed that at 4 weeks postoperative the experimental group (sub group IIA), presented few relatively thin bone trabeculae and wide marrow cavities when compared to its control counterpart (sub group IA). Such features reflect a slight delay in bone healing of the parotidectomized rats in this duration. This was emphasized by the absence of a continuous

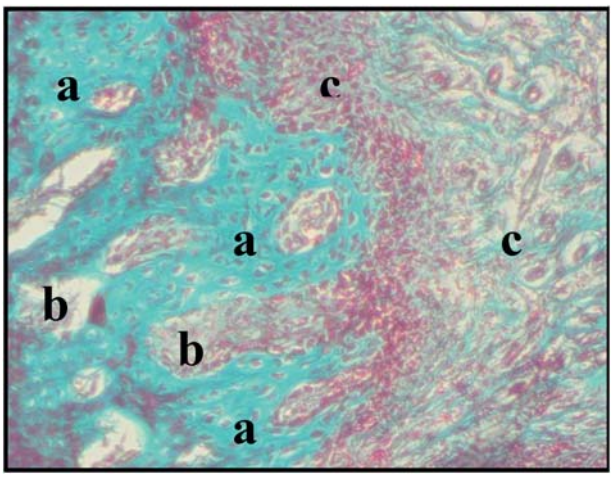

Fig. 5. Photomicrograph of **group IA** showing new collagen fibers of the newly deposited woven bone **(a)**, marrow cavities containing new collagen fibers **(b)** and the new collagen fibers scattered in the connective tissue **(c)**.

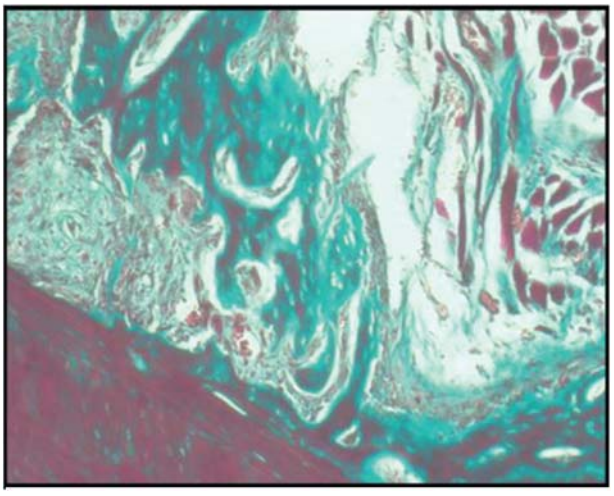

Fig. 6. Photomicrograph of sub**group IIA** showing few collagen fibers of the newly deposited woven bone (green color), the mature bone (red color) and the new collagen fibers scattered in the connective tissue. **(Masson Trichrome X 100)**.

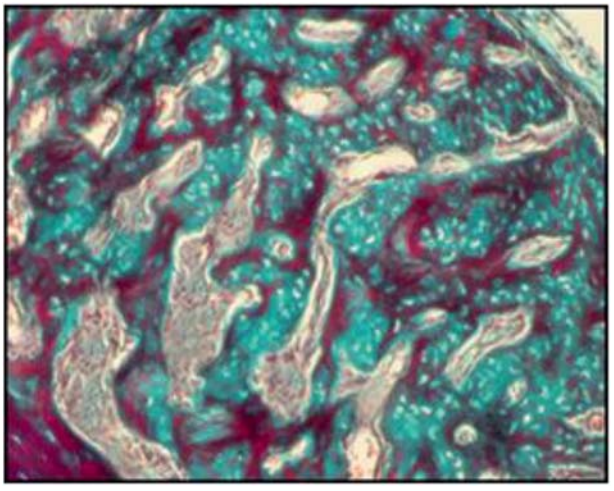

Fig. 7. Photomicrograph of subgroup IIB showing considerable amount of green areas with less red areas in between and some green areas in the marrow spaces.

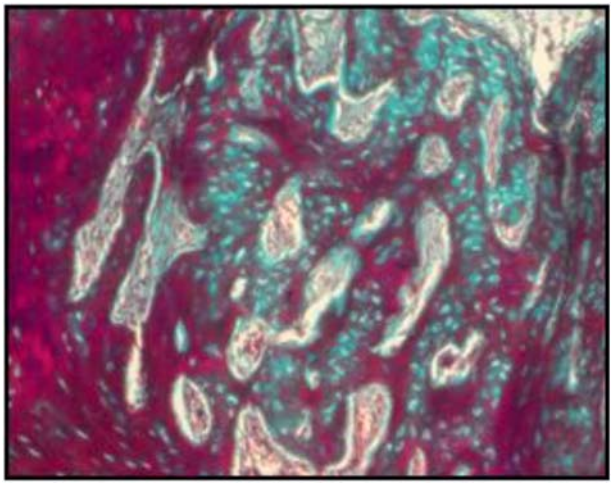

Fig. 8. Photomicrograph of subgroup IIC showing intermingling green and red areas of the bone trabeculae and green color in the marrow cavities indicates presence of collagen fibers. (Masson Trichrome X 100).

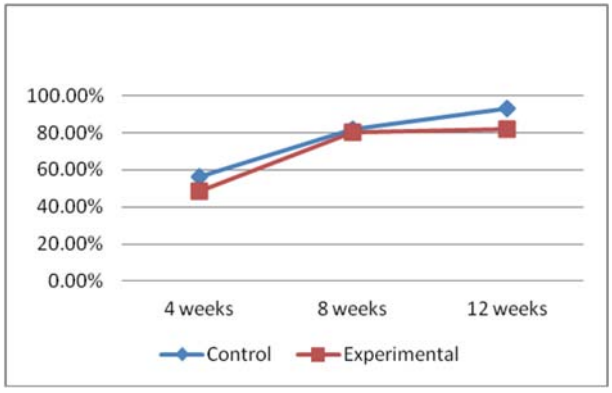

Chart 1. showing radiographic bone densities of the control and experimental groups at 4, 8 and 12 weeks post operative.

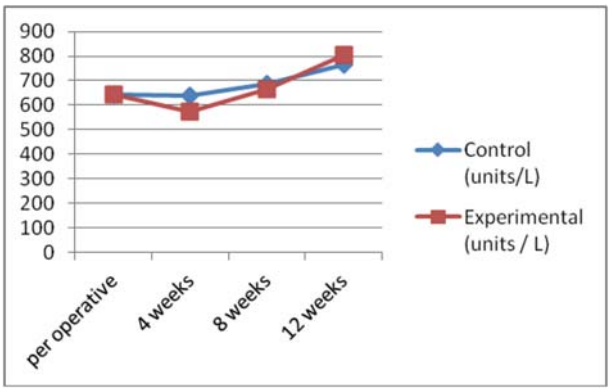

Chart 2. Showing the changes in the mean values of the serum amylase of the control and experimental groups at the pre-operative, 4 weeks, 8 weeks and 12 weeks post-operative intervals.

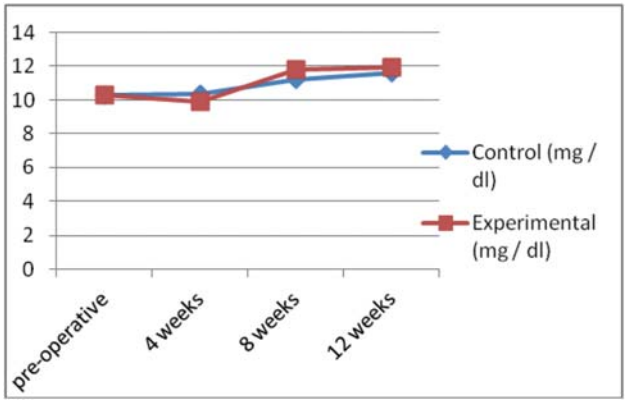

Chart 3. Showing the mean values of the serum calcium levels of the control and experimental groups at 4 weeks, 8 weeks and 12 weeks postoperative and comparing them to each other.

layer of osteoblasts in sub group IIA in contrast to its presence in sub group IA specimens.

On the other hand sub group IIA presented apparently numerous fibroblasts, such finding might reflect altered osteoblastic differentiation. This was explained by a previous study which stated that fibroblasts and osteoblasts have the same origin and can change into one another depending on stimulation or on the surrounding micro environment [3]. The irregular bone trabeculae with numerous entrapped osteocytes with large lacunae were evident in both groups regardless to the size and the extension of the newly deposited bone. It was stated that the number of osteocytes depends on the rapidity of bone formation, so they increase in woven bone and decrease in lamellar bone. Osteocytes present in large lacunae during the early stages of their transformation from active osteoblasts to osteocytes as they have a well developed golgi apparatus for collagen storage [14].

A previous study reported mild inflammatory reaction at 4 weeks postoperatively and there was a decrease in the inflammatory cells existence [2]. This comes in accordance with the control group IA of the present study where few inflammatory cells and gaint cells were sometimes detected. The presence of multi nucleated giant cells or macrophages were noticed in the injury site, as they are responsible for phagocytosis of debris and dead cell remnants [1]. On the contrary sub group II A presented numerous inflammatory cells infiltration and extravasated RBCs, these findings might reflect prolongation of the inflammatory phase of bone healing.

In the present study the amount of formed bone in the experimental group at 8 weeks (subgroup IIB) was relatively greater than their counterpart at 4 weeks (subgroup IIA) which indicated progression of bone healing process, however it is still relatively less than those of the control group (sub group IB) at the same duration. The defect of sub group IB showed continuation of bone healing process by further bone deposition towards the center of the defect and remodeling of the recently formed bone at the peripheries. It was stated that the remodeling phase is the final phase in bone healing during which the rapidly and randomly formed bone matrices become replaced by a more organized bone structure and resorption of excess callus tissue [6].

At 12 weeks the experimental group (sub group IIC) of the current study showed continuation of bone healing process, which was showed by the presence of lamellar bone at the peripheries of the bony defect. This indicated the transition between the repair phase and the remodeling phase. A picture was more or less similar to those of the control group at 8 weeks (sub group IB).

The effects of parotid gland ablation were studied on experimental fracture in albino rats, stating that there were no significant difference in the healing of the fracture sites of both the control and parotid removed groups despite there were differences in the cellularity of the healing callus of both groups [15], which in turn

coincide with the results of the current study. In the present study, the control group at 12 weeks (sub group IC) showed double band of lamellar bone and typical haversian systems in the periphery of the defect indicating success in the bone healing process and continuation of the bone remodeling phase.

No evidence of complete closure of the bony defect in either group of the present study was detected till the 12 weeks post-operative follow up interval. Therefore longer periods of evaluation more than 12 weeks to evaluate whether complete closure of the defect would occur or not. It was suggested that mandibular angle and ramus have scarce cancellous bone, the number of osteogenic cells available are small, therefore defects of any diameter at this region would not achieve complete healing [7].

The Masson trichrome stained sections at 4 weeks postoperative showed newly formed bone expressing light green stain which is indicative to the newly deposited collagen. This newly formed bone was more detected in the control than the experimental group, whereas both groups at 8 weeks showed considerable amount of newly deposited collagen fibers and variable amount of newly mineralized matrix, while the Masson trichrome stained sections at 12 weeks showed greater amount of new mineralized areas between the unmineralized collagen fibers, which was more obvious in the control group than the experimental group. It was stated that collagen fibers run in wavy branching bundles together with fibroblasts and fibrocytes. This comes in the agreement with the results of the present study at 4 weeks postoperative [9].

Analysis of histological results of the current study revealed a slight delay in bone healing of the parotidectomized rats. Such delay may be attributed to a metabolic disorder with subsequent altered digestion of food following removal of one of the major salivary glands of rats.

An alternative explanation may be that the additional surgical procedure (parotidectomy) done for the experimental subgroups may be a causative factor for delayed healing in this group. It was stated that multiple traumas may affect fracture bone healing due to accumulation of inflammatory factors and the interaction with the inflammatory processes induced by the presence of multiple injuries [8].

Comparing the statistical results of the radiographic bone densities of the control and experimental subgroups, showed better bone healing of the surgical defect in the control group than that of the experimental group, thus the surgical removal of the parotid gland had some impact on the healing of the surgical bone defect made in the rats mandibles.

The calculated p value for each group interval allowed statistical comparison and assessment of the effect of surgical removal of the parotid gland on bone healing, using the p value with statistical significance mark of $p \leq 0.05$. At 4 weeks, a p value of 0.054 indicates low statistically significant results. At 8 weeks p value was equal to 0.62 indicated a non-statistically significant difference. Finally at 12 weeks the p value was 0.05 indicating statistically significant difference between the control and the experimental subgroups. Direct correlation of the radiographic results of the current study to those from the previous studies couldn't be achieved, this was due to the wide range of variation regarding the animal models, size and site of the evaluated defects, experimental durations, and radiographic evaluation techniques and utilized soft wares.

The serum amylase enzyme results were statistically evaluated, where the p value for the 4 weeks serum amylase laboratory results was 0.173, at 8 weeks the p value was 0.151 and at 12 weeks the p value was 0.13, all of which indicating a statistically non-significant difference of the serum amylase levels of both the control and experimental subgroups along the 4 weeks, 8 weeks and 12 weeks post-operative periods.

It was mentioned that serum amylase level depressed initially then rose again to normal values over time despite absence of the glands. They suggested that other sources of the enzyme compensated for their loss [18]. Also it was stated that the serum amylase level of rats may recovered to normal values in 4 weeks postoperative and that serum amylase is mainly derived from the parotid gland, but pancreas and liver may contribute to serum amylase in compensation for the parotid glands [11].

The serum calcium results of the control and experimental subgroups were statistically evaluated to compare it using the p value. At 4 weeks, the p value was 0.061 indicating a non significant difference, while at 8 weeks the p value was 0.014 which claims that the 8 weeks results shows a statistically significant difference in the collected data between the two groups. Finally at 12 weeks the p value was 0.142 which again returns to state that the results were statistically non significant.

Analyzing the calcium level results of the current study showed that the level of serum calcium of both the experimental and control subgroups were within the normal range at 4 weeks post operative period, while at 8 weeks and 12 weeks the serum calcium levels slightly rose but still close to the normal level, this was thought to be due to the increased absorption from intestine to meet the mineralization demand in these stages. This comes in accordance with a study which stated that calcium reaches the skeleton by being absorbed from the diet in the gastrointestinal tract, where the absorbed dietary calcium then enters the extracellular fluid space and becomes incorporated into the skeleton through the process of mineralization of the organic matrix of bone (osteoid tissue) [13].

7. Conclusions

From the present study, it could be concluded that bilateral parotidectomy in albino rats resulted in delayed bone healing. It was associated with an initial drop in serum amylase level at 4 weeks, however serum amylase level was self-compensated at 8 and 12 weeks of postoperatively. It didn't significantly influence serum calcium level. The role of parotid salivary gland in osteogenesis is mostly mediated through its endocrinal function. Further investigations targeting the endocrinal role of the parotid gland seems essential.

References

[1] Alexander KA, Chang MK, Maylin ER, Kohler T, Muller R, Wu AC, et al. Osteal macrophages promote in vivo intramembranous bone healing in a mouse tibial injury model. J Bone Min Res 2011;26:1517–32.
[2] Ali AA, Mohammed SS. Histological evaluation of intrabony defect repair induced by white ordinary Portland cement (WOPC). J Bagh Coll Dent 2011;23(3):44–9.
[3] Andrade ZA, Oliveira-Filho J, Fernandes ALM. Interrelationship between adipocytes and fibroblasts during acute damage to the subcutaneous adipose tissue of rats: an ultrastructural study. Braz J Med Biol Res 1998;31(5): 659–64.
[4] Ardakani FE, Navabazam A, Fatehi F, Ardekani MD, Khadem S, Rouhi G. Histologic evaluation of chitosan as an accelerator of bone regeneration in microdrilled rat tibias. Dent Res J Isfahan 2012;9(6):694–9.
[5] Bancroft. In: Bancroft JD, Gamble M, editors. Theory and practice of histological techniques. fifth ed. Philadelphia: Churchill Livingstone publishers; 2002.
[6] Buckwalter JA, Einhorn TA, Bolander ME, Cruess RL. Healing of the musculoskeletal tissues. In: Rockwood CA, Green DP, Bucholz RW, Heckman JD, editors. Rockwood and Green's fractures in adults. fourth ed. Philadelphia: LIPPINCOTT-RAVEN; 1996. p. 261–304.
[7] Chin VKL, Shinagawa A, Naclerio-Homem M. Bone healing of mandibular critical size defects in spontaneously hypertensive rats. Braz Oral Res 2013;27(5):423–30.
[8] Claes L, Ignatius A, Lechner R, Gebhard F, Kraus M, Baumgärtel S, et al. The effect of both a thoracic trauma and a soft tissue trauma on fracture healing in a rat model. Acta Orthop 2011;82(2):223–7.
[9] El-Fayomy S, El-Shahat A, Omara M. Healing of bone defects by guided bone

regeneration (GBR). Egypt J Plast Reconstr Surg 2003;27(2):159–66.
[10] Hirano T, Manabe T, Tobe T. Cellular alterations of parotid gland of rats with acute pancreatitis induced by cerulean. Int J Pancreatol 1991;10:217–27.
[11] Ikeno K, Ikeno T. Effects of prolonged parotid duct ligation, parotidectomy and acute hepatitis of rats on amylase activity. Arch Oral Biol 1991;36(3):183–8.
[12] Kalfas IH. Principles of bone healing. Neurosurg Focus 2001;10:7–10.
[13] Kini U, Nandeesh BN. Physiology of bone formation, remodeling and metabolism. 2012. http://dx.doi.org/10.1007/978-3-642-02400-9_2.
[14] Kumar GS. Orban's oral histology and embryology. 13 th ed. 2011. p. 213–4.
[15] Martínez MPA, Gámez FN, Melón ERM. Effects of parotid gland ablation on experimental fracture: callus and its microvascular modifications. Bone circulation bone necrosis 1990:53–8.
[16] Parrilla C, Saulnier N, Bernardini C, Patti R, Tartaglione T, Fetoni AR, et al. Undifferentiated human adipose tissue derived stromal cells induce mandibular bone healing in rat. Arch Otolaryngol Head Neck Surg 2011;137(5):463–70.
[17] Peacock M. Calcium metabolism in health and disease. Clin J Am Soc Nephro 2010;l5:23–30.
[18] Proctor GB, Asking B, Garrett JR. Serum amylase of non-parotid and non-pancreatic origin increases on feeding in rats and may originate from the liver. Comp Biochem Physiol 1991;98:631–5.
[19] Robling AG, Castillo AB, Turner CH. Biomechanical and molecular regulation of bone remodeling. Annu Rev Biomed Eng 2006;8:455–98.
[20] Roland B. Anatomy and ultrastructure of bone histogenesis, growth and remodeling. Chapter Bone 2008:300–50.
[21] Struck MB, Andrutis KA, Ramirez HE, Battles AH. Effect of a short term fast on ketamine xylazine anesthesia in rats. J Am Assoc Lab Anim Sci 2011;50:344.