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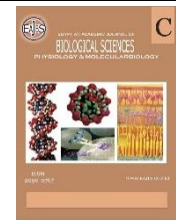
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Serum Alkaline Phosphatase As A Biomarker for Bone Damage After Immediate Basal Implant Placement In Jawbones of Beagle Dogs

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ABSTRACT

This study aimed to find the level of bone damage in the jaw bones after multiple basal implant placements in both upper and lower jaws by having the serum level of alkaline phosphatase. **Materials and methods:** Ten beagle dogs were involved in the first stage of the study when the first and second premolars in both maxillary and mandibular segments of the left side of the mouth were extracted and followed by immediate implant placement of screw-designed basal implants. A preoperative and immediate postoperative blood collection was done initially, and a waiting period of four months was enough to have a mature bone formation in the peri-implant areas, that was the time for late postoperative blood collection. The blood samples were centrifuged to have sera for alkaline phosphatase examination. **Results:** no significant difference was detected between the preoperative and the immediate postoperative groups while a significant difference was detected in comparison with the late postoperative group. **Conclusions:** no significant jawbone damage can be correlated to the basal implants even with multiple teeth extractions and multiple basal implants' immediate placement procedure.

INTRODUCTION

The recent decades came with researchers studying relatively new implant designs to restore lost teeth, such designs could be more acceptable from patients' point of view; since those new implants can be immediately loaded and used for functional and esthetical purposes. These implants are known as basal implants and represent a good treatment according to some researchers who found after years of follow-up and peri-implant tissue examination that these implants could preserve the surrounding tissues rather than lose them (Hahn, 2007). When some researchers tried to compare the immediate loading procedure using the conventional, basal implants with the delayed loaded implants from patients' comfort and success rate point of view. The results did not support the idea of a better success rate of a specific study group at the time that some patients were complaining about more pain and discomfort in the immediately loaded basal implants group (Garg *et al.*, 2017).

These findings are subjective, the time that no objective criteria were followed to find more about the degree of damage related to the type of implant we are using.

The role of alkaline phosphatase in the diagnosis of bone tissue condition was related to the serum level of this enzyme, to indicate the significance of this marker -in comparison with other markers- in highlighting possible bone damage (Hussain *et al.*, 2024). Some researchers were more specific in their investigation about the correlation between bone damage and the bone-specific alkaline phosphatase level, to have further information about the degree of bone damage and the related bone-specific together with total serum alkaline phosphatase levels (Muljačić *et al.*, 2010). In a different study, the same team of researchers found a relationship between callus volume and alkaline phosphatase level, just to prove a further correlation between bone healing and serum level of alkaline phosphatase (Poljak-Guberina *et al.*, 2020).

There are four different types of serum alkaline phosphatase: liver-bone-kidney type, intestine type, placental type, and microbial variation. It is found in the bones, hepatocytes, kidneys, spleen, placenta, prostate, leukocytes, and small intestine. Alkaline phosphatase and its isoenzymes are used to diagnose disorders of the liver function, bone structure, digestive tract, and parathyroid glands. High levels can be detected in circumstances characterized by a rise in osteoblast activity, hepatobiliary disorders with intra- or extrahepatic cholestasis, septicemia, chronic inflammatory bowel disease, or thyrotoxicosis. The rise in alkaline phosphatase catalytic activity distinguishes all kinds of cholestasis, particularly obstructive jaundice; it is also enhanced in skeletal system disorders such as Paget's disease, fractures, and cancers (Massányi *et al.*, 2020).

This study aimed to find the level of bone damage in the jaw bones after multiple basal implant placements in both upper and lower jaws by having the serum level of alkaline phosphatase before, immediately after and months after the healing process.

MATERIALS AND METHODS

As soon as agreement (reference number: VM2022/1820UD) from the Ethical Board of the College of Veterinary Medicine/University of Duhok had been given, the first stage of the study was started using ten healthy, adult Beagle dogs. They were brought from the Dogs' yard at the municipality of Zakho and were fitted in the Animals Investigation facility at the College of Veterinary Medicine/ University of Duhok. The animals were maintained in individual cages in a (12:12 lightness/darkness series) and treated with anti-helminths drugs, vaccinated with antivirals, and provided with three meals of mixed diet, dry food and three times a day changes of tap water.

The implants were bought from Demirtas implant company, the DE|TECH screw-designed basal implants, the length of the fixture was 12 mm, and the abutment was 7.5 mm. The diameter of the compression screw implants was 3.5 mm while the diameter of the bicortical screw basal implants was 3.6 mm.

The animals were anaesthetized under general anaesthesia just to collect blood (preoperative sample). A portable dental X-ray machine (Eighteenth Hyperlight G) and a dental sensor (size 2) were used to have periapical radiographic examinations of the first and second premolars both in the maxilla and mandible; for preoperative area examination and implant area measurements with the Nanopix software application. The maxillary and mandibular first and second premolars in the left side of the mouth were extracted after careful soft tissue separation with the dental probe. In the case of the first premolar simple extraction procedures were carried out using dental forceps, whereas in the case of the second premolar dissection with a high-speed dental turbine handpiece was necessary to have each root extracted individually. The extraction was followed by cleaning of the dental sockets with Lucas's bone curette and irrigation with normal saline

solution, and then the implants were placed immediately into the sockets of the left maxillary and mandibular first and second premolar extraction sites, where each tooth socket received an implant. Then immediate postoperative blood collection was done (postoperative sample).

A waiting period of four months was necessary for peri-implant mature bone formation and gingival attachment. Then blood was collected for the third time, after anaesthetizing dogs under general anaesthesia (late sample). The blood was collected in a serum tube, and centrifuged for five minutes at 3000 rpm to have the wanted serum in the serum tube.

The serum was kept in the freezer before being sent to the laboratory for chemical analysis with the Cobas c111 analyser used for in vitro diagnostic purposes.

Twelve dogs were included in the first stage, and a series of six dogs were excluded; since the freezer power was off for four containers on the weekend days, whereas two dogs were excluded in the second stage; one of which died and the other one was reported with full mouth implant failure. Such exclusion led to having a sera of six dogs only being included in the study.

Statistical Analysis:

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One-way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. Post hoc tests are an integral part of ANOVA. We used ANOVA to test the equality of at least three group means using the F test but this test does not identify which particular differences between pairs of means are significant. Hence, we should use post hoc tests to explore differences between multiple groups' means. The chi-square test was used to assess the significant differences among proportions. $P < 0.05$ is considered statistically significant.

The related reference was: SAS.2010.SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.

RESULTS

The sera were examined in different stages, the first stage contained preoperative and postoperative sera. While the second stage contained the late serum samples.

The related results of the serum alkaline phosphatase test are listed in Table 1.

Table 1: The results of serum alkaline phosphatase tests

Dog number	Alkaline phosphatase/preop	Alkaline phosphatase/postop	Alkaline phosphatase/late
1	16	21	18
2	39	36	34
3	61	56	15
4	87	86	9
5	59	59	27
6	39	43	16

No significant difference between the preoperative and postoperative results of the alkaline phosphatase was detected. In

contrast, the late alkaline phosphatase results significantly differed from the first-stage test results, see Table 2, and Figure 1.

Table 2. The least significant difference and standard error of the mean of serum alkaline phosphatase in each stage. Means with a different letter are significantly different ($P < 0.05$).

Groups	Mean \pm Standard Error
Alkaline phosphatase/preop	50.17 \pm 9.95a
Alkaline phosphatase/postop	50.17 \pm 9.13a
Alkaline phosphatase/late	19.83 \pm 3.70b
LSD	24.36

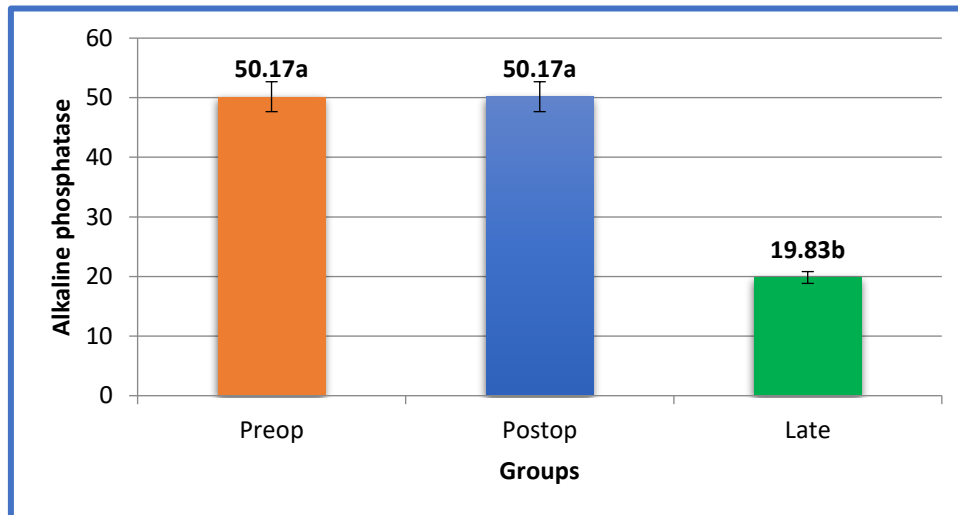


Fig. 1. Serum alkaline phosphatase levels in each stage

DISCUSSION

Bone formation activity can be assessed using serum biomarkers such as alkaline phosphatase. Measurement of this during fracture healing improves the accuracy of determining the bone healing stage, allowing for early diagnosis in individuals at risk of developing delayed union or non-union (Priyanka Verma, 2022).

In a study about the role of the elevated level of serum alkaline phosphatase, in patients presented with a history of chronic renal failure and were hospitalized for hemodialysis, it was found that the high serum alkaline phosphatase is associated with a high mortality rate and can be used as an indicator of possible related hip fracture. The same patients who responded well to the treatment and had improved conditions were associated with lowered levels of serum alkaline phosphatase (Maruyama *et al.*, 2014).

Recent recommendations advised further role of the alkaline phosphatase as an agent to relate the possible tissue damage in

oral diseases or even specific osseous tissue growth follow-up (Shetty *et al.*, 2017). Other researchers used the specific type of alkaline phosphatase to correlate different stages of bone healing to the body's enzymatic response, they found that the level of serum bone-specific alkaline phosphatase in mandibular fracture healing fluctuates between 3 and 6 weeks. It is proportional to the amount of callus developed at the fracture site after three weeks and inversely proportional to the amount formed after six weeks. As a result, bone-specific alkaline phosphatase is a determinant of callus formation in individuals with mandibular fractures (Taofeek *et al.*, 2023) which can support the idea of using this enzyme as a biomarker to check bone damage in the oral and maxillofacial region, a reason that helped us to use this enzyme to test the extent of bone damage caused by more invasive types of implants (basal implants). The bone-specific alkaline phosphatase serum level used to be a good indicator of bone damage that is directly proportional to the serum level of alkaline

phosphatase according to the *Muljacic et al* findings (Muljačić *et al.*, 2010). The significance of serum alkaline phosphatase as a biomarker in oral tissue damage research can agree with our and some other researchers' findings like *Sharma et al* who could correlate the serum alkaline phosphatase level with the degree of periodontal damage caused by chronic periodontitis (Sharma *et al.*, 2023) though we cannot agree with the degree of sensitivity of the serum alkaline phosphatase as a biomarker, that research was indicating.

Our findings could not indicate any bone damage that can be significant enough to increase the level of serum alkaline phosphatase in between the preoperative and immediately postoperative tests; since the basal implants used were not destructive to a degree that can be compared to the bone fracture. Anyway, the significant decrement in the serum level of the alkaline phosphatase after four months of the waiting period between the first and the second stages can be attributed to the improved lifestyle -whether medical care or healthy nutrition- of those dogs and that can agree with the *Nestor et al* findings about the effect of life conditions on the serum alkaline phosphatase level (Nestor *et al.*, 2006).

Conclusions

- The alkaline phosphatase serum level was not significantly different preoperatively and immediately postoperatively; because the bone damage was not high enough to provoke such marker response.
- Alkaline phosphatase is a good marker to detect significant bone damage. Still, it is not a highly sensitive marker to detect relatively less jawbone damage caused by minor oral surgical procedures.

Declarations:

Ethical Approval: The study was approved by the Ethical Board of the College of Veterinary Medicine/University of Duhok (no. VM2022/1820UD).

Conflict of interest: The authors declare no conflicts of interest.

Authors Contributions: All authors contributed equally, and have read and

agreed to the published version of the manuscript.

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Availability of Data and Materials: The data presented in this study are available on request from the corresponding author.

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