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FACILITATING OSTEOGENESIS OF CHITOSAN WITH AUTOGENOUS BONE MARROW (EXPERIMENTAL STUDY ON RABBITS)

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ABSTRACT

Bone quality is the result of a complex relationship between the intrinsic properties of the materials that comprise the bone matrix mineralization, bone mass and the spatial distribution of the bone mass. Chitosan has been shown to be suitable bone replacement material. To evaluate the accelerating effect of chitosan on the bone regeneration process and assessing by CT Scan were conduct this study. Several important biological effect of chitosan has been characterized, these are high osteoinductivity, osteointegrability and gradual biodegrability that make it a good candidate for bone regeneration. Materials and Methods: 20 rabbits of both sex were enrolled in this study, two monocortical defects were created on Mandible, one considered as control and the other implanted with chitosan, other two monocortical defects were created on Tibia on the same animal. Post-operative follow up date 7,14,21and 28 Days. C.T. scan was used as parameter for bone density measurement. Results: showed that non-significant difference at Day7 and14 in Mandible and significant at Day21 and 28 compared to control, While non-significant at Day 7 in Tibia and significant at 14 and 21 post-operatively with highly significant at Day28 compared to control. Conclusion: Chitosan has ability to osteogenesis when it is used alone and the process of osteogenesis was facilitating when it is mixed with Bone marrow.

KEY WORDS: Chitosan. Bone Marrow. Osteogenesis.

INTRODUCTION

Chitosan is an aminopolysaccharide, produced from the deacetylation of chitin obtained from shells of exoskeletons such as (shrimp and crustaceans), It has many biological applications like antimicrobial effect, antitumor activity, antioxidant activity hemostatic effect, facilitating of bone formation and used as bone graft material (Daniel et al., 2016).

Bone defect may developed in various surgical procedures in clinical dentistry whether it is benign or invasive that of malignant lesions. the bone should be restored in patient as soon as possible, the limitation of use autogenous bone graft in terms of availability may be more desirable especially when it is fill the requirement such as scar size, post-operative pain and improve patient recovery on of the best materials which full fill these requirement is chitosan (Fatemeh et al., 2012). It is one of synthetic bone substitute material that overcomes the limitation of Autogenous and allograft bone graft, in addition of biocompatibility, nontoxicity, biodegrability and high bioactivity (Raafat and Sahl, 2009; Majeti,2000). The biodegradability of chitosan depends on the degree of deacetylation and molecular weight, chitosan toxicity is rest on its molecular weight, degree of deacetylation, and route of administration (Chae et al.,2005). Chitosan with degree of deacetylation values higher than 35% displayed low toxicity, while a degree of deacetylation value under 35% caused dose-dependent toxicity (Aiba, 1992).

Chitosan that used in the present study was produced by Shaanxi Top Pharm Chemical Co.,LTD. which was a white powder with De-acetylation more than 90% and moisture less than 10%.

Bone morphogenic proteins (BMP) have an important role in osteogenesis. Chitosan can be used to develop microparticles to be used as a controlled delivery system for BMP. both chitosan and BMP promote cell growth and

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differentiation, they act as a better scaffold material for adhesion, proliferation, and differentiation of pre-osteoblasts in comparison with controls (Jasmin et al., 2009).

MATERIALS AND METHODS

Twenty rabbits were elected weight (2000-2500 g), they were randomly classified into four groups five for each and sacrificed at day 7, 14, 21 and 28 post-operatively. They were anaesthetized intramuscularly with Ketamine (50mg/kg) and Xylazine (20mg). The surgical operation done on Mandible and Tibia.

In the Mandible, a skin incision was made extraorally along the inferior border of mandible to expose the underlining bone. Two monocortical holes was performed with trephine bur (3mm) width and (2mm) in depth with (5mm.) in between by slow speed handpiece with copious irrigation. The first defect implanted by chitosan. The second is control, then the flap re-approximated and sutured. Fig.(1)

In Tibia, a full- thickness flap was reflected to expose the underlining tibia bone. two monocortical holes was performed with trephine bur with (3mm) in width and (4mm) depth with (5mm.) in between until reach the marrow space. the first left empty and considered as control, the second hole implanted with Chitosan, then flap was reapproximated and sutured. Fig.(2)

Post-operation care by gentamicin sulfate ampule (80mg) and gentamycin ointment externally of both mandible and tibia immediately following the surgery till day 7 post operatively. animals scarified at 7,14,21 and 28 post-operative day and speciments of mandible and tibia were planned for CT scan for bone density measurement. Fig.(3)

RESULTS

Bone density was measured by taking the mean of three points at defect site for each anatomical plane , sagittal plane, coronal plane, and the transverse plane.

In Mandible, and at the end of day 7 and 14, it is shown no significant difference in chitosan the mean was (134.83 ±13.97) and the mean was (251.67±7.44) when compared to control the mean was (124.7±11.03) (251.67±7.44), (P≤0.549) (P≤1.00) respectively.

At the end of day 21 and 28 there was significant difference in bone density, at the end of day 21 it was in chitosan (509.07±14.07) compared to control was (366.23±10.11) and (P≤0.038), and at end of day 28 it was in chitosan (824.33±24.41) compared to control was (476.47±14.11) and (P≤0.044). (Table 1)

In Tibia, and at the end of day 7, there was no significant increase in bone density, in control the mean was (127.27±11.27), while chitosan the mean was (134.3±11.9), (P≤0.674). At the end of day 14 there was significant difference in bone density, control one the mean was (256.83±7.58) and chitosan the mean was (336.43±9.93), (P≤0.02). At the end of day 21 and 28, there was highly significant increase in bone density, in control the mean was (373.4±10.33) (538.43±15.94) and in chitosan the mean was (653.63 ± 18.08) (803.73±53.38), (P≤0.001) (P≤0.00) respectively.
(Table 2).

DISCUSSION

Chitosan in Mandible

This study was performed to investigate, evaluate and compare the effect of chitosan when applied on bone marrow in bone regeneration process by using CT scan.

At the end of day 7 and 14 there was non-significant differences in bone density at mandible and this findings was in agreement with a study done by Kjalarsdóttir et al that demonstrated on rats and sacrificed after surgery (7, 10, and 14), bone was investigated by using CT scan, No significant new bone formation was observed in the implants at any time points (Kjalarsdóttir et al., 2019). Differentiating osteoblasts progress through three general phases: first, an early proliferative period that continues for several days after confluence, with the cells exiting the cell cycle between days 3 and 7; secondly, a period of collagen deposition and extracellular matrix maturation between days 8 and 14; and finally, terminal differentiation characterized by mineralization of the mature matrix after 15 days (Beck et al., 2000). While at the end of day 21 and 28 early high measure of bone density in chitosan defect compared to control, these result were in agreement with Chatzipetros et al that showed chitosan has ability to induce bone formation at 21 and 28 days post-operatively when it is applied in a defect created on rat calvaria models, CBCT scores showed that statistically significant at 4 weeks compared to control group (Chatzipetros et al., 2018). This is due to the ability of chitosan to differentiated to

mesenchymal cells to osteoblasts (bone forming cells) and facilitate formation of bone, trail to osteogenic activity on created defects in animal models (Li et al., 2012).

Chitosan in Tibia (within Bone Marrow)

At the end of day 7 there was non-significant differences in bone density between chitosan and control. Interestingly the expression of bone morphogenetic proteins BMP2, BMP 4, and BMP 7 are relatively low in the initial healing period when compared with later healing phases (woven bone formation, remodeling). BMP2 and BMP7 are the growth factors have been clinically approved for the treatment of non-unions (Jasmin et al 2010).

At day 14, there was significant difference in bone density between chitosan and control in Tibia, which agree with findings of Alsarrag and Yassen, which was a study on rabbits showing the effect of chitosan on osteogenesis, by extraction of lower anterior teeth and socket were implanted with chitosan, in the second group extraction done but without implantation with chitosan as control groups. The area under the grafted material gradually filled with newly bone formation adjacent to bone periphery of the defect cavities, this tendency became more apparent with increasing time of implantation at (2, 4 weeks) post-operatively, this is due to the ability of chitosan to enhance bone formation (Alsarrag and Yassen, 2008).

At day 21 and 28, there was significant difference, the results were agree with Nandi et al, a study on rabbits, to investigate effectiveness of chitosan with insulin like growth factor-1 and (BMP-2), Radiologically, there were evidence of radio-density in defect of chitosan with (IGF-1) and chitosan with(BMP-2), as compared to chitosan alone (Nandi et al .,2012). Osteoblasts develop from pluripotent mesenchymal stem cells that have the potential to differentiate into adipocytes, myocytes, chondrocytes, and osteoblasts under the direction of a defined suite of regulatory transcription factors. Osteoblast differentiation is controlled by the master transcription factor RunX2 (runt-related transcription factor 2) (Franceschi et al., 2003). Previous studies proposed that chitosan nanofibers may induce Runx2 gene expression in osteoblasts via the bone morphogenetic protein signaling pathway (Hung et al., 2010). Chitosan reported to promote the differentiation of mesenchymal stem cells into osteoblasts and facilitated the formation of bone in vitro (Klokkevold et al l996). and also had osteogenenic activity on artificially made bone defects in animal models (in vivo) (Li et al., 2012).

CONCLUSION

It can be concluded that Chitosan alone can facilitate new bone formation and maturation and decreased healing period, and when added to BM. New bone formation increased within less period.

List of Tables

Table (1): Measurements of Bone Density at Mandible by C.T scan.

Days / Mandible	Day 7 Mean ± SD	Day 14 Mean ± SD	Day 21 Mean ± SD	Day 28 Mean ± SD
Control	124.7 ± 11.03	251.67 ± 7.44	366.23 ± 10.11	476.47 ± 14.11
Chitosan	134.83 ± 13.97	253.67 ± 7.44	509.07 ± 14.07	803.73 ± 53.38
P value	0.549	1.00	0.038	0.044
≤ 0.05			*	*

* Significant difference at P≤0.01

Table (2): Measurements of Bone Density at Tibia by C.T scan

Days / Mandible	Day 7 Mean ± SD	Day 14 Mean ± SD	Day 21 Mean ± SD	Day 28 Mean ± SD
Control	127.27 ± 11.27	256.83 ± 7.58	373.4 ± 10.33	538.43 ± 15.94
Chitosan	134.3 ± 11.9	336.43 ± 9.93	653.63 ± 18.08	824.33 ± 24.41
P value	0.674	0.02	0.001	0.00
≤ 0.05		*	*	*
≤ 0.001			**	**

*Significant difference at P≤0.01
** Highly Significant difference at P≤0.001

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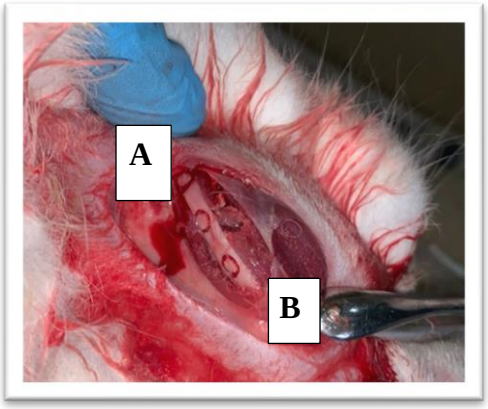

Fig. (1): Created holes along the Mandible, **A**:control **, B:** Chitosan

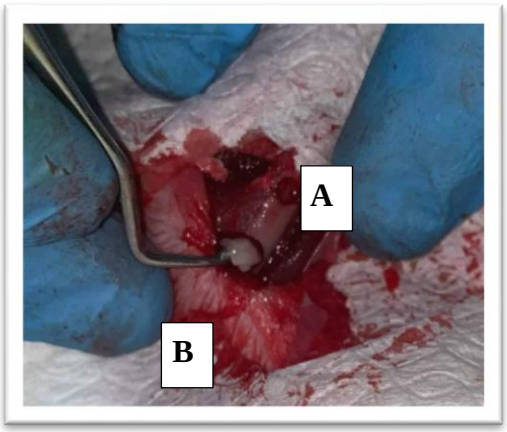

Fig. (2): Created holes along the Tibia **A**:control **, B:** Chitosan+BM.

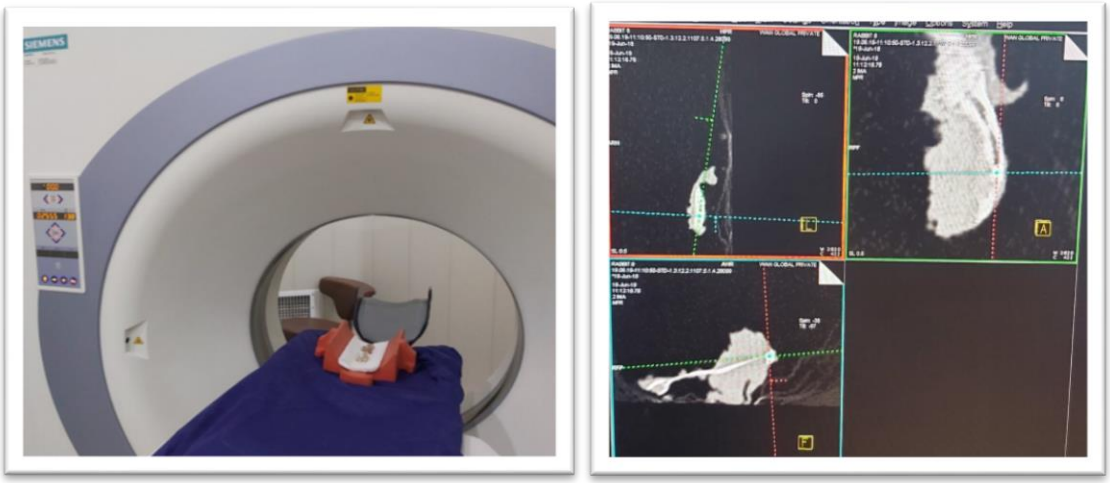

Fig.3 A-CT scan equipment**, B-** Measuring the bone density

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