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RANDOMIZED CONTROLLED CLINICAL STUDY EVALUATING DENTAL IMPLANT PRIMARY STABILITY, RADIOGRAPHIC BONE DENSITY AND OSTEOPONTIN EXPRESSION IN THE REGENERATED ALVEOLAR BONE IN POST-EXTRACTION SOCKETS TREATED WITH VITAMIN D3

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ABSTRACT

Purpose of the study: Evaluating the influence of combination of vitamin D3 with particulate bovine xenograft when placed in post-extraction sockets on the newly formed bone quality.

Primary outcome: Implant primary stability

Secondary outcomes: Radiographic bone density and immunohistochemical expression of osteopontin in newly formed regenerated bone

Subjects and methods: Patient with non-restorable maxillary anterior tooth or single root premolar that require extraction and dental implant. Test group: 13 extraction sites received particulate xenograft mixed with vitamin D3. Control group: 13 extraction sites received particulate xenograft alone. After 6 months implants were placed. Implant primary stability, radiographic bone density, immunohistochemical osteopontin expression in grafted sites were evaluated.

Results: A significantly higher mean value of both primary stability and bone density were recorded in test group.

Conclusion: Taking in considerations the limitations of the current study, the addition of vitamin D to particulate xenogenic bone graft enhanced newly formed bone density, implant stability and is associated with increased expression of OPN level in grafted sites.

KEYWORDS : Tooth Socket, Vitamin D, augmentation

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INTRODUCTION

Alveolar ridge resorption is a common consequence of tooth loss. (Al-Askar et al 2013) The majority of vertical and horizontal alveolar bone dimensional changes occur within the first 3-6 months following tooth loss with the greater changes occurring in the width. (Tan et al 2012) A systematic review showed that the alveolar bone dimensional changes after extraction can be minimized effectively by alveolar ridge preservation procedure and the type of grafting material affects the outcomes. (Bassir et al 2018)

Procedure-related and patient-related factors play a role in the complex osseointegration process. (Beer et al 2003). Quantity and nature of the bone at the edentulous area together with the host response affects osseointegration. (Insua et al 2017).

Implant stability is a clinical assessment for successful osseointegration, and it involves primary and secondary stabilities (Natali et al 2009). The primary stability refers to the mechanical interlocking of the implant within the surrounding alveolar bony structure without implant movement immediately after insertion while the secondary stability is determined by the bone remodeling and healing procedure and is referred as being biological stability. (Greenstein et al 2008, Natali et al 2009). A strong correlation exists between primary and secondary stability and any impairment in primary stability may negatively affect the osseointegration (Roos et al 1997). Primary stability is affected by the type of bone, design of implant and surgical procedure while secondary stability is related to osseous remodeling, surface treatment and the initial stability. (Javed & Almas 2010, Javed et al 2013, Karl et al 2018). Implant stability can be assessed by many methods: reverse torque test, cutting resistance analysis (Johansson & Strid 1994, Friberg et al 1995), periotest (Olive & Aparicio 1990,) or resonance frequency analysis (RFA) (Sul et al 2002)

Originally bone mineral density (BMD) is considered equivalent to bone quality but ideally bone quality is denoted by multiple factors that affect the bone strength and its resistance to fracture. Usually bone mineral density is used by clinicians to objectively determine the quality of bone. (Wakimoto et al 2012). Poor density bone is associated with increased bone resorption and disturbance in the healing sequence around the implants and can be considered a significant risk factor for failure of the implant. (Jaffin & Berman 1991, Herrmann et al 2005, Herrmann et al 2016). Foreseeable data regarding implant stability can be obtained by bone density measured on CBCT. (Tatli et al 2014). The new CBCT machines create good quality images , which can be manipulated by programs on computer to provide precise measurements of BMD. (De Oliveira et al 2008, Benavides et al 2012)

Osteopontin (OPN) is a main non-collagenous protein in the matrix of mineralized tissues. (Nagata et al 1991a,b, Fisher & Fedarko 2003) It is synthesized by osteoblasts, odontoblasts, and osteocytes. (Staines et al 2012). In alveolar bone and teeth, osteopontin represents a part of the organic extracellular matrix (ECM). (Sodek et al 2000, Lin et al 2020). OPN plays a role in many physiological and pathologic events like remodeling of bone, healing of wound and metastasis in tumors. (Sodek et al 2000, Ishii et al 2004). Many studies documented the OPN crucial role in regulating bone resorption, bone formation and mineralization. (Fisher & Fedarko 2003, Holm et al 2014) At the meeting line of original and newly regenerated bone and at the osseous surfaces facing cells, high concentration of OPN was noticed. (Mckee & Nanci 1995). Hypophosphatemia, Hypocalcemia (Sodek et al 2000), Inflammatory mediators , Glucocorticoids and 1,25-dihydroxy vitamin D3 (Pike et al 2014) lead to upregulation of OPN.

Important role is played by Vitamin D3 in regulating calcium metabolism and bone minerals homeostasis. It is crucial for the control of intestinal calcium absorption, calcium /phosphate level maintenance for bone formation and parathyroid hormone functions. The majority of body cells expresses vitamin D receptors (VDRs). (Jones 2014)

Vitamin D stimulates osteoclastic activity and also increases the osteoblastic production of extracellular matrix proteins such as collagen and osteopontin. (Reid et al 2014) VDRs are expressed in osteoblasts where interactions with vitamin D3 regulate many genes affecting bone maturation and mineralization. (Haussler 2013). The intake of vitamin D and calcium can promote increased bone density and healing of fractures. (Lips P and Schoor NM 2011)

Experimental animal studies showed that addition of vitamin D to grafts can improve the outcomes of reconstructive bony surgeries (Hong et al 2012, Alexander et al 2015) Also treating the surface of the implant with vitamin D yields promising results on osteoblastic cell functions. (Satué et al 2015) Moreover, vitamin D low level in blood can negatively affect bone healing around implants and vitamin D supplementation can enhance osseointegration. Healing of peri-implant bone can be enhanced by vitamin D administration. (Apostu et al 2017)

So far relatively few animal studies evaluated the influence of vitamin D on dental implants and peri-implant bone quality (Choukron et al 2014, Javed et al 2016, Salomo-coll et al 2016, Sundar et al 2023)

In the study by Amr AEH 2019, combination of vitamin D3 with bovine particulate xenograft in the management of deficient ridges enhanced volumetric bone formation in favor of the vitamin D3 group but the nature of the gained bone was not evaluated either radiographically or histologically.

Purpose of the study

Evaluating the influence of combination of vitamin D3 with bovine particulate xenograft* when placed in post-extraction sockets on the newly formed bone quality.

Primary outcome: Implant primary stability

Secondary outcomes: Radiographic bone density and immunohistochemical expression of osteopontin in newly formed regenerated bone

SUBJECTS AND METHODS

Research question:

Does treatment of the post-extraction sockets with vitamin D3 mixed with particulate xenograft capable of enhancing the quality of regenerated alveolar bone within the socket?

Research hypothesis:

The alternative hypothesis of this randomized clinical trial suggests that adding vitamin D to xenografts in alveolar ridge preservation will enhance the quality of regenerated bone in the socket compared to using xenografts alone.

PICOTS:

Patient/problem: Patient with non-restorable maxillary anterior tooth or single root premolar that require extraction and dental implant.

Intervention: Grafting of the extraction socket with particulate xenograft mixed with vitamin D

Comparator: Grafting of the socket with particulate xenograft alone

Outcomes: Implant primary stability, radiographic bone density, immunohistochemical osteopontin expression

Time: 6 months after grafting

* Cerabone, 100% pure bone mineral of bovine origin, botiss biomaterials

Setting: Faculty of dentistry, Ain Shams University

Before starting the study, approval for the study design and steps was taken from the ethics committee faculty of dentistry Ain Shams University. (Approval number: FDASU-Rec PC 022465). It is a double blinded study with a 1:1 allocation ratio.

Study details was explained to all patients and approval was taken and all patients had all the rights to withdraw any time during the study. All the study data were treated with a high degree of privacy and confidentiality.

Sample size calculation:

To evaluate dental implant primary stability, radiographic bone density and osteopontin expression in the regenerated alveolar bone in post-extraction sockets treated with vitamin D3, independent test or an equivalent non-parametric was used for comparison between 2 groups. According to Abtahi et al in (2012) the effect size between the 2 groups was recorded as Cohen's d=1.3

By G power statistical power Analysis program (version 3.1.9.4), ***A total sample size (n=22; subdivided to 11 per group)*** was sufficient to detect a ***large effect size (d) = 1.3,*** with an actual power ***(1-β error) of 0.8 (80%)*** and a significance level ***(α error) 0.05 (5%)*** for two-sided hypothesis test. (table 1)

TABLE (1) Sample size calculation

Effect size	α error	Power (1-β error)	Total sample size	Sample size per group
1.3	0.05	0.8	22	11

$$n = \frac{2(Z_{\alpha} + Z_{1-\beta})^2 \sigma^2}{\Delta^2}$$

n means the sample size.

Zα, Z is a constant (=1.96 for α error 0.05 (5%) with two-sided effect)

For Z1-β, Z is a constant set by convention according to power of the study as shown below:

Power	80%	85%	90%	95%
Value	0.8416	1.0364	1.2816	1.6449

σ = standard deviation (estimated)

Δ = difference in effect which is required (estimated effect size).

Patient selection:

Inclusion criteria:

20-35 years-old male or female, systemically free based on burket's health questionnaire (Glick et al 2008). Patients with maxillary single root anterior tooth or premolar that require extraction. Extraction socket with sound bone walls; socket type 1, this was detected initially by preoperative cone beam computed tomography (CBCT) and confirmed clinically after tooth extraction. (Elian et al 2007)

Exclusion criteria:

Teeth with periapical and/or periodontal infection, smokers, patients taking systemic vitamin D supplements or multivitamins and vulnerable subjects.

Patient randomization and grouping:

Four patients were added to the sample size (22 patients) to have total 26 participant in the study suggested by the power analysis. This was performed to compensate for any dropouts, but fortunately all the 26 patients (13 in each group) completed the study without any withdrawals, complications, or failures.

Blinding of the participants and data analyst was achieved (double blinded). Patients were randomly allocated by computer (www.Randomizer.org) in ratio 1:1.. Allocation concealment was followed using sequentially numbered opaque sealed envelopes.

Group A (test group): 13 extraction sites received particulate bovine xenograft mixed with vitamin D3.

Group B (control group): 13 extraction sites received particulate bovine xenograft alone

Study procedures:

Methylcellulose was mixed with the solvent to prepare methylcellulose in situ gel. The mix was heated at 55 °C and using a shaker a clear solution was formed.

Aqueous vitamin D3* was then added and dissolved completely to make a homogenous gel. Gel was placed in sterilized syringes. Storage in a cool dry area after sterilization (Hong et al 2015). 25 ml gel is formed of 80 I.U vitamin , 2 g hydroxyl propyl methyl cellulose and 10g water.

At the initial visit complete patient data and medical history were recorded followed by detailed examination clinically and by CBCT. Periodontal debridement and hygiene instructions were given two weeks before extraction. The nature of the study was discussed with all patients and they signed the consent.

The first surgery involved atraumatic extraction and grafting of the socket. The second surgery was performed six months later and involved harvesting core biopsy from the regenerated bone and implant placement. Atraumatic extraction was performed using periotomes and luxators** under local anesthesia. Any soft tissue remnants in the socket were then removed by bone curette followed by saline irrigation. The test site received particulate xenograft*** hydrated with vitamin D3 gel (test gel) 10 minutes before application in the socket followed by collagen cone**** on top of the graft then figure eight polypropylene sutures*****. The control site received xenograft alone (hydrated with normal saline) followed by collagen cone on top of the graft then figure eight polypropylene sutures. Sutures were removed 10 days later.

Postsurgical instructions and medications:

Oral Amoxicillin - Clavulanic acid****** 1gm BID 5days, Metronidazole******* 500mg BID 5 days . Oral Analgesic******** TID 3days. Chlorhexdine******** oral rinse BID for 5 days.

The second surgery for implant placement and harvesting of core biopsy was performed 6 months after grafting. Before drilling for implant , core biopsy was harvested by trephine bur with inner diameter 2mm******** from the site of future dental implant then sent to the oral pathology lab for bone histological and immunohistochemical study. Prosthetic procedures were completed 4 months after implantation.

Assessment:

Assessment of the regenerated bone nature was performed via measurement of the primary stability using the resonance frequency analysis RFA, bone density and osteopontin expression.

RFA involves attaching a small metallic rod to the implant fixture then magnetic pulses are produced by the Ostell® device toward the rod. The device records the vibration degree of the rod. (Abdulhameed et al 2018).

Radiographic bone density was detected using a CBCT performed 6 months after grafting before implant placement. Taking in consideration that the intervention in our study was grafting of empty sockets with the same type and form of bone grafts

* (active material manufactured by "medical union pharmaceuticals MUP"

** (Hufriedy , USA)

*** Cerabone, Botiss biomaterial GmbH

**** Collacone, Botiss biomaterial GmbH

***** Egyprolene, Egypt

****** (Hibiotic, Amoun, Egypt)

******* Amrizole, pharco, Egypt)

******** (voltarin, Novartis, Egypt)

******** (Orovex-H, Macro, Egypt)

******** Hufriedy, USA

in both groups , and the core of the study was bone quality evaluation rather than dimensional changes thus there was no need for additional immediate postoperative CBCT after grafting for assessment of density or dimensions.

The patients were scanned using i-CAT Next Generation (i-CAT; Imaging Sciences International, Hatfield, PA).

A scan was taken of the maxilla (scan dimensions of 6 × 17 cm) for 40 seconds with the following setting of the iCAT—voxel size: 0.2 mm; gray scale: 14 bits; focal spot: 0.5 mm; image detector: amorphous silicon flat panel; image acquisition: single 360° rotation.

The images were transformed to (DICOM) and then the i-CAT vision software was used to make the radiographic evaluation in ideal dimly lit viewing conditions (15.6 inch HD LED) at the highest resolution setting (1680 *1050). Magnification, contrast, and brightness changes were used to make precise measurements.

On the implant screen, the arch was drawn so that it paths through the root canal at the CEJ level. Cross-sectional image data were derived from the axial-source raw data. The display format for all images were set to be 3x1 and magnified to the region of interest (ROI).

The three cross-sectional images of the ROI were viewed with spacing of 1.8mm.

The gray level representing the density of bone in the ROI was measured using the HU (Hounsifield units) statistics tool as follows: two squares of the same size were drawn on each of the three cross sectional images representing almost the whole length of the graft (figure 1). Caution was taken so that they were centered only within the cancellous regenerated bone and not involving any of the cortical bones. The software automatically displays the mean of the gray level representing the density in each area. Then, the average of the total 6 measurements on the three cross sectional images were recorded for further analysis to represent the mean of the dentistry of this ROI.

Immunohistochemical assessment of osteopontin bone marker expression and histological evaluation of the harvested core biopsy were performed.

Formalin 10% was used for bony specimen fixation for a couple of days, decalcification was then done by EDTA 5% PH 7.0 for 14 days

Tissues were placed in paraffin wax. Longitudinal sections (5-mm thick sections) were then prepared. Hematoxylin and eosin (H&E) and Masson's Trichrome were used for staining. Immunohistochemical staining was performed as follows: Blocks were cut (thickness 4 micrometers), and then sections were mounted on positively charged glass slides. Sections were deparaffinized and rehydrated in alcohol. Sections were immersed in citrate and treated in a microwave before staining. The Peroxidase-antiperoxidase immunostaining using the biotin-streptavidin system was applied, 3% hydrogen peroxide was applied to the section to block endogenous peroxidase. Immunolabelling of the sections using primary monoclonal

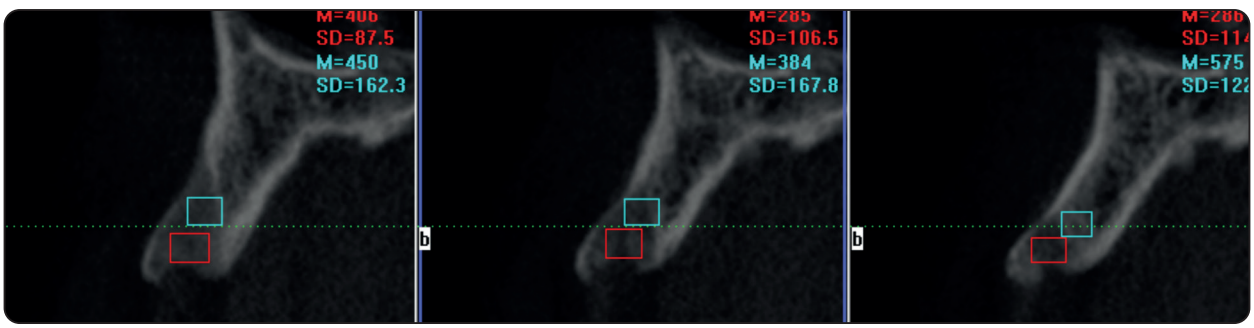

Fig. (1) CBCT Image representing the method of measurements of the dentistry in the ROI.

lyophilized antibody (clone OP3N, Vision biosystems Novocastra™ Laboratories, England) and then incubation at room temperature. sections were covered by link antibody after rinsing , then streptavidin labeling antibody. After rinsing, diaminobenzidine chromogen was added to the sections followed by counterstain. Sections were dehydrated, cleared and mounted.

Statistical study was done using the (SPSS) version 20. Data were checked for normality by checking the data distribution using Kolmogorov-Smirnov and Shapiro-Wilk tests. Comparisons between groups with respect to normally distributed numeric variables were done by independent t test.

Pearson correlation test was used to measure the strength of the linear relationship between bone density and implant stability.

All p-values are two-sided. P-values ≤0.05 means significant

RESULTS

Demographic analysis

Mean age was **32.08±2.72** in test group and **31.92±2.9**3 in control group. No significant difference between the mean age values in different groups (p=0.891), (Table 2). Regarding the **Gender,** Test group consisted of 84.6% females and 15.4% males, while control group consisted of 76.9% females and 23.1% males. In both groups no significant difference regarding gender distribution was found (p=0.619), (Table 3). **Tooth distribution** is presented in Table (4). Difference between groups regarding tooth distribution was not significant (p=0.627).

TABLE (2) Age (years)

		Test	Control	t value	P value
Age(years)	Mean ±SD	32.08±2.72	31.92±2.93	0.139	0.891 ns
	Min- Max	28-36	27-36		

Significance level p≤0.05, ns= Non-Significant

TABLE (3) Gender distribution

Gender / n (%)	Test	Control	X^2 value	P value
Males	2 (15.4%)	3 (23.1%)	0.248	0.619 ns
Females	11 (84.6%)	10 (76.9%)		

Significance level p≤0.05, ns= Non-Significant

TABLE (4) Tooth distribution

Tooth / n (%)	Test	Control	X^2 value	P value
maxillary central	7 (53.8%)	8 (61.5%)	0.933	0.627 ns
maxillary lateral	4 (30.8%)	2 (15.4%)		
maxillary premolar	2 (15.4%)	3 (23.1%)		

Significance level p≤0.05, ns= Non-Significant

Comparison between the groups

Bone density: A significantly higher mean value was recorded in test group (569.54±158.44), in comparison to control group (328.62±138.76). The mean difference between groups was (240.92±58.41), Confidence intervals [120.37 to 361.48]. This difference was statistically significant (p=0.000) (Table 5, Fig 2).

Implant stability: A significantly higher mean value was recorded in test group (70.31±4.82), in comparison to control group (59.62±4.94). The mean difference between groups was (10.69±1.91), Confidence intervals [6.74 to 14.64]. This difference was statistically significant (p=0.000). (Table 5, Fig 3)

Correlation between stability and density in each group

In Test and control groups: a statistically significant very strong positive correlation was noted between density of bone and stability of implant (R=0.896, p=0.000), (Table 6, Fig.4,5)

Overall: a statistically significant very strong positive correlation was noted between bone density and implant stability (R=0.938, p=0.000), (Table 6, Fig. 7).

TABLE (5) Bone density and Implant stability and groups comparison (independent t test)

		Mean	Std. Dev	Median	95% Confidence Interval for Mean		Min	Max	t value	P value
					Lower Bound	Upper Bound				
Bone Density	Test group	569.54	158.44	610.00	473.79	665.28	205.00	682.00	4.12	.000*
	Control group	328.62	138.76	306.00	244.76	412.47	165.00	633.00		
Implant Stability	Test group	70.31	4.82	70.00	67.40	73.22	58.00	76.00	5.59	.000*
	Control group	59.62	4.94	60.00	56.63	62.60	50.00	67.00		

Significance level p≤0.05, *significant

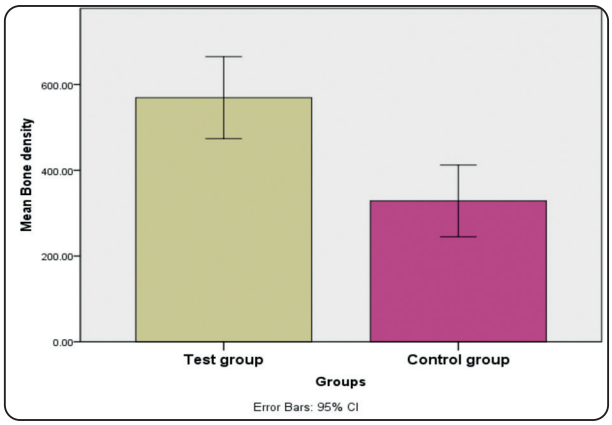

Fig. (2) Bar chart of bone density

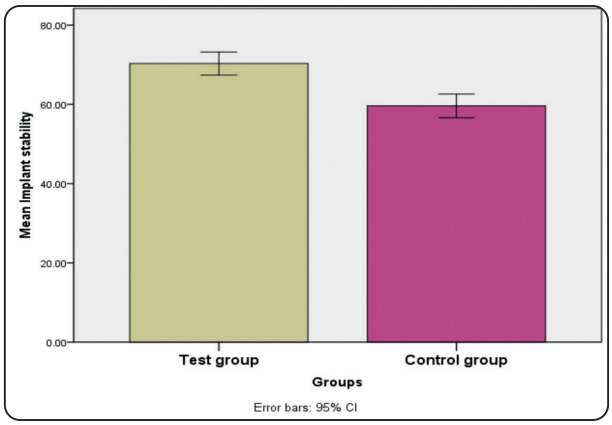

Fig. (3) Bar chart of implant stability

TABLE (6) Correlation between density and stability (Pearson's correlation test)

	Test group	Control group	Overall
Pearson Correlation (R)	.896**	.910**	.938**
P value	.000	.000	.000
Interpretation	Very strong +ve	Very strong +ve	Very strong +ve

Significance level p≤0.05, *significant

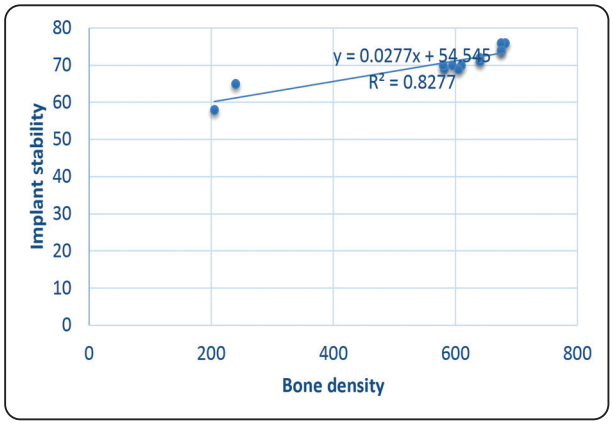

Fig. (4) Scatter plot showing strong positive correlation in test group

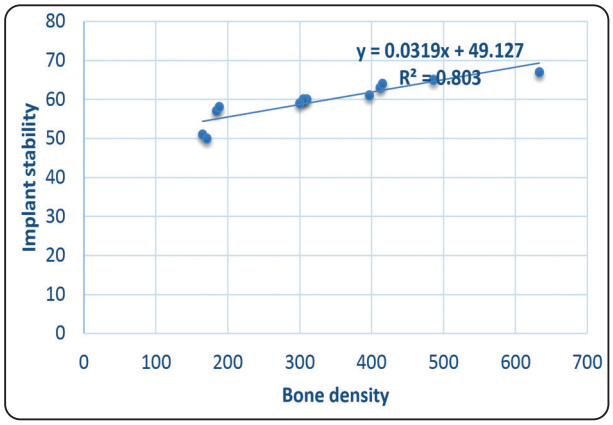

Fig. (5) Scatter plot showing strong positive correlation in control group

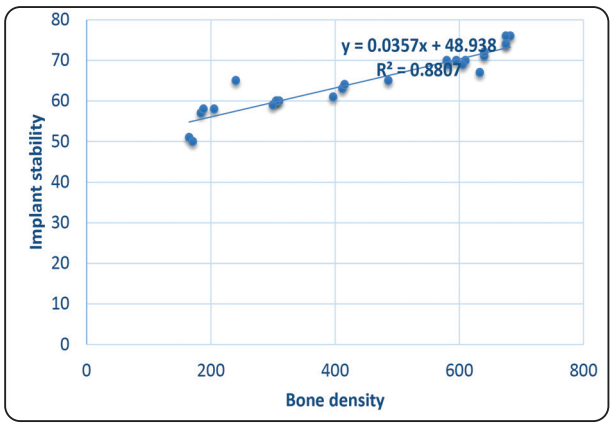

Fig. (7) Scatter plot showing overall strong positive correlation

Histological and immunohistochemical assessment: (Figure 8)

The test group showed new bone formation. Active process of forming bone was detected via the prominent rimming of osteoblasts in woven bone. Remodeling of bone was detected by the apparent reversal lines. Large number of osteocytes were seen in lamellar bone. Remaining particles of graft materials were detected as deep stained areas and seen integrated with the regenerated bone. Prominent dense capillaries in highly vascular tissues fill the medullary spaces. A small number of inflammatory cells were detected. The control group showed also some new bone, woven, lamellar bone, osteocytes. Trabeculae are seen but not interconnected. Also, some graft residues are present.

Masson's trichrome slides results are similar and agreeing with the features seen on the aforementioned H&E examination. .

Regarding immunohistochemistry results, osteopontin was highly expressed in the test sites in compare to the control sites. Osteopontin was expressed more in osteocytes, osteoblasts, around osseous trabeculae and in spaces of bone marrow.

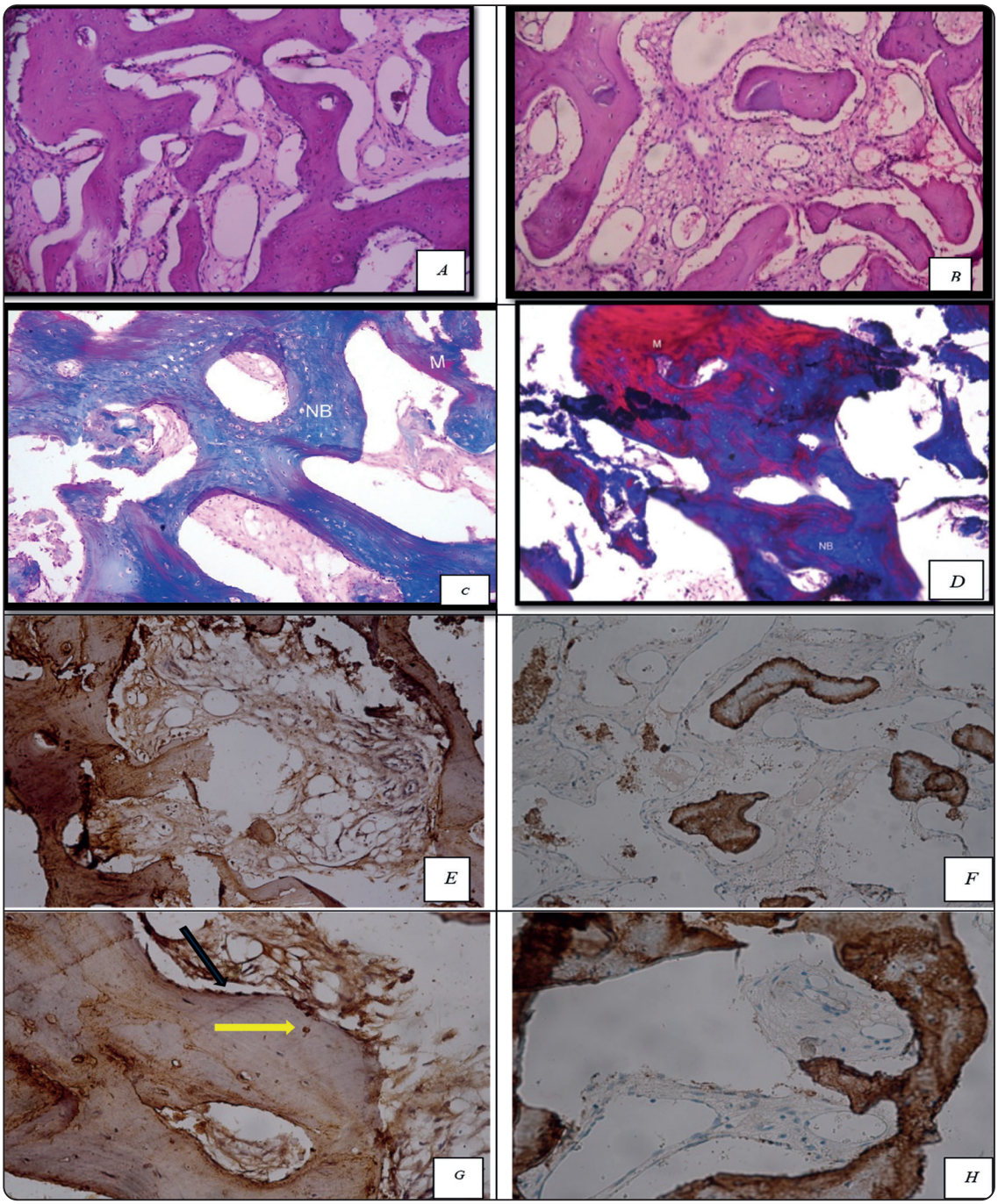

Fig. (8) Photomicrograph of Test group showing large osteoid interconnected trabeculae and highly cellular stroma which contain active plump of osteocyte in lacuna . active osteoblasts around the trabeculae (A) while the Control group showing medium sized non-interconnecting trabeculae with plump of osteocyte in lacuna (B) (H&E stain x10). Photomicrograph of Test group showing large amount of new bone trabeculae with large number of osteocytes in lacunae and surrounded by few capillaries. (C), while Control group showing less new bone trabeculae (D) (M. Trichrome stain x20) . Immunohistochemical assessment revealed increased expression of osteopontin in the test sections (E) than control sections (F).(osteopontin x 20). positive expression of osteopontin in osteoblasts, osteocytes, surrounding bone marrow cells and tissue in test group (G) (osteopontin x40). Control group bone marrow tissue showed less expression of the osteopontin (H) (osteopontin x40).

DISCUSSION

Different techniques and biomaterials have been documented for alveolar ridge preservation ARP with a general agreement that ARP reduces post-extraction bone resorption but cannot totally prevent it (Horvath et al 2013). Different bone substitutes allow preservation of the ridge after tooth removal but the nature of the regenerated bone are variable and sometimes the graft shows interference with the normal healing process (Bassir et al 2018)

The use of growth factors, hormones and vitamins with the bone substitute in bone grafting procedures can yield enhanced regenerative results (Sohn et al 2017).

The current research was performed to assess the quality of alveolar bone regenerated using vitamin D3/xenograft clinically, radiographically and immunohistochemically.

Stability of the implant was evaluated in the present study by using Ostell® being simple, easy and resulting in objective reliable data clinically (Sennerby & Meredith 2008, Satwalekar et al 2015). Proper implant stability is assumed when ISQ values is greater than 65 while poor primary stability and increased risk of implant failure is indicated when ISQ value is below 45. (Ramakrishna &Nagar 2007) . Being highly important in influencing the primary stability, bone quality can affect the whole treatment plan. (Molly 2006, Wilmes et al 2008).

Bone mineral density (BMD) in implantology can be measured by computerized axial tomography (CT) and Cone beam computed tomography (CBCT). (Jeong et al 2013). In our study the CBCT was selected to assess the density of the newly regenerated bone . Many studies reported that CBCT can be considered a radiation dose-reducing technique when compared to medical CT in dentistry. (Benavides et al 2012). The use of CBCT for BMD measurement in the oral cavity have been validated. (Parsa et al 2015)

Hounsfield unit values obtained from MDCT (multi-detector CT) is the standard method in clinical practice to assess bone mineral density (BMD). In the case of CBCT, data are often corrupted by many factors such as cone artifacts, detector inhomogeneity, and scatter leading to inaccurate estimates of bone density (Liu Y, et al., 2013).

Since the quantitative calibration of CBCT is highly dependent on the size, shape, and density of the imaged object, the calibration and correction methods were either difficult to implement or not generally valid for different applications (Liu Y, et al., 2013).

Several observations were found in different studies regarding the variation in the gray level on CBCT images. A study by Nackaerts O et al., 2011 found that under the exact same exposure conditions and positioning, the intensity values are quite reproducible. HU values of the same material measured on CBCT are not comparable when they are placed in different relative positions (Liu Y, et al., 2013). Gray values obtained from CBCT are influenced by the position of target objects in the FOV that leads to unreliable estimation of bone density (Araki K. and Okano T, 2013).

On the current study, the gray levels representing the density of a specific ROI obtained from the CBCT were used. Although these values are relative, do not represent the true density and cannot be used in another study, they still give an idea about the bone density alternations related to the graft material used and can be used in comparing between the two study groups in the current study.

Regarding choosing osteopontin as an immunohistochemical marker to be measured in our study is based on previous studies that documented the important role played by osteopontin in bone remodeling and regulation of bone formation, resorption and mineralization (Fisher & Fedarko 2003, Holm et al 2014). Studies showed that OPN-deficient osteoclasts have no ability to perform bone resorption and when exogenous OPN was added

to these osteocalsts, enhanced osteoclastic motility was noticed (Chellaiah & Hruska 2003, Singh etal 2018)

Eligibility criteria were followed to avoid variables that could affect the results. No patients with any systemic disease were involved in the study to avoid any factors that could affect the healing and liability for infections. All the patients were non-smokers to avoid any negative effect of smoking on grafting procedure (Levin et al 2005).

Regarding the statistical analysis for radiographic bone density and comparison between groups, higher mean bone density was noticed in test group with statistically significant difference between groups. So far as we know, no past clinical human trials tested locally delivered vitamin D in ARP with bone density evaluation of the gained tissue. The results obtained can be caused by vitamin D added to the xenograft in test group . Previous studies documented that vitamin D regulate bone mineralization and maturation controlling both osteoblasts and osteoclasts (Haussler 2013). Supplementation with vitamin D and calcium can enhance healing of fractures and the bone density in humans (Dawson-Hughes et al 1997).

Regarding the statistical analysis for implant primary stability and comparison between groups, higher mean primary stability was noticed in test group with statistical significant difference between groups. Taking into consideration that the same surgical technique and same implant design were used in both test and control sites , thus the significant difference in mean primary stability between groups is related to the difference in the obtained bone quality. (Rue et al 2021)

The higher density and higher primary stability obtained in the test group are matching with previous researches which concluded that enhanced bone regeneration can be obtained when mixing vitamin D with the graft in bone regenerative procedures (Gogolewski et al 2006, Sundar 2023). Another study tested the influence of dietary vitamin D on implant osseointegration in rats and showed that it can improve stability of implant (Dvorak et al 2012). Moreover, some studies evaluated the coating of implant with vitamin D and showed increased implant-bone contact, enhanced osseointegration thus increased implant stability. (Cho et al 2011, Salomo – Coll etal 2016)

Immunohistochemical staining showed higher expression of osteopontin (OPN) in test group than the control group. Osteopontin is a main non-collagen protein found in bone matrix, it is expressed by many human cell types. The expression of OPN has basically been explained as an indicator of bone formation. Our results were consistent with several studies which revealed that vitamin D3 has a notable direct influence on osteoblast growth and differentiation, via more expression of alkaline phosphatase, osteocalcin and osteopontin. The effect of vitamin D3 also might be due to its influence on human mesenchymal stem cells (hMSCs) differentiation towards osteoblast-associated characteristics and the increased expression of osteogenic markers. So in the current study the higher expression of OPN in test group confirms that vitamin D3 showed higher positive effect on bone formation by allowing osteoblastic differentiation, leading to more new bone formation than the control group.

This was in aggreement with researches that proved that OPN expression is upregulated by 1,25 dihydroxy vitamin D3 (Pike et al 2014) and that it exerts a prominent effect in remodeling of bone and is concentrated at lines of old and newly formed bone (Mckee and Nanci 1995). Also the increased OPN expression in the vitamin D group was in accordance with a study carried in 2019 by Mercan and Turer which evaluated the effect of Vitamin D intraperitoneal administration in osteoporotic rats (single dose 50.000 mg/kg) on the bone formation in grafted bony defects. Their results revealed enhanced new bone formation histopathologically and increased OPN level by immunohistochemical analysis.

CONCLUSION

Taking in considerations the limitations of the current study, the addition of vitamin D to particulate xenogenic bone graft enhanced newly formed bone density, implant stability and is associated with increased expression of OPN level in grafted sites.

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