Clinical Evaluation of Biochemical Marker and Mineral Nutritional Factor in Mandibular Implant Over-Denture Cases

Yaser M. Alkhiary

King Abdulaziz University, Faculty of Dentistry, Prosthodontics Department Jeddah, Saudi Arabia
alkhiary@gmail.com

Abstract: Vitamin-D and calcium deficiencies are considered as a major clinical and global health problem and specifically dentistry when planning an implant therapy in extremely atrophied alveolar bone with poor quality in elderly patients. Ten female completely edentulous patients with atrophied edentulous mandible were asked to participate in the study. Each participant received two endosseous titanium implant in the mandibular symphysis area and a mandibular overdenture with Locator® attachments. Subjects were divided into two groups: Five subjects were given vitamin-D and calcium oral supplements (Study Group) and the other 5 subjects did not receive any supplements (Control Group). Analysis of bone marker; serum Calcium, Osteocalcin and Alkaline Phosphate and the level of Nitric Oxide (NO), Tumor Necrosis Factor-alpha (TNFα) and Matrix Metalloproteinase-8 (MMP-8) were performed for each subject immediately before implant placement, after 1, 3 and 6 months of implant placement. There was a statistical significant decrease in the levels of MMP-8, NO and TNFα in the study group compared to the control group but there was a significant increase in the level of serum calcium, osteocalcin and alkaline phosphate in the study group compared to the controls. Vitamin-D and Calcium supplement administration could aid in the success of implant treatment. [Yaser M. Alkhiary**. Clinical Evaluation of Biochemical Marker and Mineral Nutritional Factor in Mandibular Implant Over-Denture Cases**. *J Am Sci* 2012;8(12): 507-513]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 71

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1. Introduction

Vitamin-D deficiency is an epidemic worldwide and Saudi Arabia as well as other countries is no exception. Studies conducted on a population in Saudi Arabia reported that the prevalence of vitamin-D deficiency was 50% to 80% [1,2].

Vitamin-D is a fat-soluble vitamin obtained from three sources. Endogenous, where Vitamin-D is synthesized in the skin and is induced via ultraviolet radiation. It is also be obtained exogenously through dietary sources or through supplements that are converted to 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D through utilization of ultraviolet radiation that stimulates its synthesis from a cholesterol precursor in the epidermis. Vitamin-D has been shown to regulate musculoskeletal health by mediating calcium absorption and mineral homeostasis [3]. Evidence has demonstrated that Vitamin-D deficiency may place subjects at risk not only for low mineral bone density/osteoporosis and osteopenia but also at risk of infections and chronic inflammatory disease [4,5]. Vitamin-D may play a beneficial role for oral health, through its direct effect on bone metabolism by its ability to function as an anti-inflammatory agent and stimulate the production of anti-microbial peptides [6-8]. Vitamin-D is also involved in the regulation of immune responses by enhancing macrophage chemotaxis and phagocytic capacity. Vitamin-D also inhibits the expression of monocytic inflammatory cytokines including IL-1, IL-6, IL-8, IL-12 and TNFα [9-12].

Tooth loss and alveolar Residual Ridge Resorption (RRR) are significant oral health problems. The pathogenesis of tooth loss, and RRR involves both local and systemic factors. The local biomechanical masticatory problem, related to denture wearing, has been proposed as a possible cause for RRR. On the other hand, continued RRR occurs in edentulous individual without dentures. Several studies have showed a relationship between oral bone loss and systemic bone loss, suggesting that loss of alveolar bone is a manifestation of osteoporosis [13]. The severe resorbed alveolar ridge is treated either by vestibuloplasty, ridge augmentation or by implants. However, the excessive and continuous bone resorption may compromise the treatment with implants and hence, affect bone augmentation procedure [14].

Successful osseointegration of dental implant is influenced by several factors, including but limited to bacterial, occlusal (mechanical factors) and systemic factors. One of the systemic factors is nutritional deficiency. Unhealthy (nutritionally deficient) oral tissues will not provide the satisfactory foundation for successful implant. One such deficiency Calcium and Vitamin-D which are recommended for implant patients [15]. Therefore, the identification and management of

nutritional deficiencies could be considered a useful adjunct to successful implant treatment.

Most of scientific work focusing on peri-implant soft tissue has increased dramatically in recent years. This work was focused on examining the morphologic feature of epithelium and the connective tissue around implants. However, limited data exist on their function, structural requirement as barrier tissue, the immune and inflammatory cell infiltration [16].

Tumour Necrosis Factor-α (TNFα), a nonglycosylated protein, is considered a pro-inflammatory cytokines that possess multiple effects and bone resorptive properties. TNF-α plays a major role in the immune response especially in cell-mediated cytotoxicity by inducing cytotoxic T-lymphocytes differentiation, enhance monocyte cytotoxicity and stimulate lymphocyte-activated killing. It also has marked effects on epithelial cells and at high concentrations is considered cytotoxic. However, the prolonged release of TNFα has been reported to cause stem cell damage and epithelial atrophy [17,18].

Acute rejection is mediated by humoral and cellular immune mechanisms and is characterized by an intense inflammatory cell infiltrate and progressive destruction of the grafted organ. The rejected organ is the source of these increased end products of L-arginine-nitric oxide synthesis. Activated macrophages are thought to be a major source of nitric oxide in immune-mediated tissue reaction. Evidence of TNFα and nitric oxide-mediate epithelial injury in graft versus host disease has been presented [19,20].

Several studies found that the conventional method of periodontal indices used for evaluating peri-implant soft tissues are unreliable and are unfit for clinical evaluation in dental implants and can not be used as clinical marker for bone loss [21,22].

Matrix metalloproteinase-8 (MMP-8), also called collagenease-2, or neutrophil collagenease) is an indicator of extracellular matrix breakdown during pathologic process because of its exclusive pattern in the inflammatory condition. They play a major role in peri-implant tissue destruction during peri-implantitis. MMP-8 is a member of zinc and calcium dependant neutral endoproteinasees with 28 member (MMP1-MMP28). They play an important role in the normal physiological process such as morphogenesis, reproduction and tissue remodelling [23,24]. Since this issue is really important for osseointegration of dental implant ,so our study was conducted to evaluate the effects of Ca-VitD supplements on the levels of TNFα, NO, MMP-8 and Alkaline phosphatase in edentulous subject who received implants mandibular overdenture.

2.Materials and methods:

Ten female completely edentulous patients with atrophied edentulous mandible were selected from the Prosthodontic Department, King Abdulaziz University, Faculty of Dentistry. Their age ranged from 45 to 60 years. Exclusion criteria were: any systemic disease, poor oral hygiene and history of chemotherapy or radiotherapy. The study design was explained to the patients and a signed consent was acquired. The study was reviewed and approved by Research Ethics Committee at the Faculty of Dentistry, King Abdulaziz University.

After collecting all the required diagnostic records for each patient, acrylic complete maxillary and mandibular dentures with bilateral balanced occlusion were fabricated for each patient. Radiographic surgical stent was fabricated from clear heat acrylic replica of the constructed mandibular complete denture.

All patients received two endosseous titanium implants. They were placed at bone crest (Prima Connex, Keyston, Burligton, MA, USA) in the mandibular symphysis area. After two weeks, the conventional upper and lower dentures were used and relined with soft lining material, with the appropriate thickness to ensure adequate relief over the implant tissues. Then patients were allowed to use their dentures. Clinical and radiographic x-ray (panoramic and periapical) evaluations were performed to ensure implant integration. Then patients were randomly divided equally into two groups (Study and control group, each of five). The study group received Calcium 500mg + Vit. D3 400IU Caplet (BioCal-D, Arnet Pharmaceuticals, Davie, FL, US.A) orally twice daily after two weeks of the implant insertion, for six months. The control group did not receive any supplements.

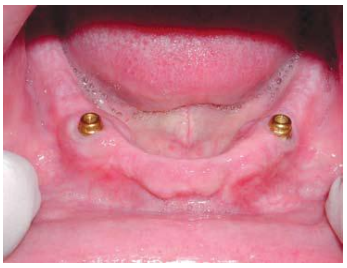

Figure 1 Two Locator attachments was placed in the mandibular arch four months after implant placement

Locator attachments (Zest, Escondido, CA, USA) were incorporated in the base of patient's mandibular denture (Fig.2) as follow:

The Locator® abutment was inserted supra-gingivally and torqued at 35 Ncm. The white block-out spacer was placed over the head of the Locator® abutment. Then a Locator® denture cap with black processing male was inserted into each

Locator® abutment, leaving the white block-out spacer beneath it. Prepared a recess in the denture base to accommodate the protruding Locator® denture Cap Processing male assembly (There must be no contact between the denture and the processing cap). An autopolymerized acrylic resin mix (Acrostone Cold Cure Acrylic Resin, Acrostone Co., England) used to bond the processing cap in the denture. Small amount of acrylic resin was applied to the recess of the denture base and around the processing cap. The denture was inserted in the patient mouth; guide the patient into occlusion, maintaining a proper relationship with the opposing arch while the acrylic sets. The denture was removed, cleaned and examined for the orientation of the attachment inside the denture base and the white block out spacers was discarded. Any excess acrylic was removed and the denture base finished before changing to the final replacement male. The black processing male removed from the metal processing cap with the male removal tool. The male seating tool was used to insert a Locator® replacement male into the empty metal processing cap. Then the denture was inserted and patient was motivated for oral hygiene procedure and then scheduled for radiographic and biochemical follow up.

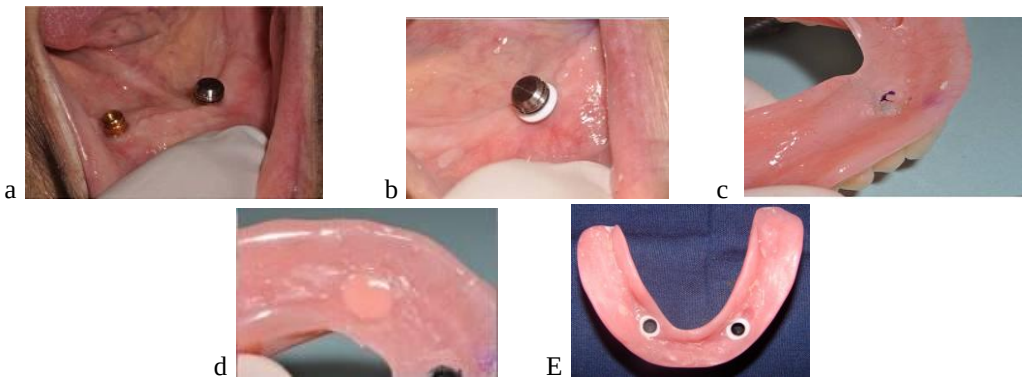

Fig.2: Locator attachments incorporated in the base of patient's mandibular denture

Biochemical evaluation:

Sulcular fluid sample collection was performed as follows: The surface of the implants were dried gently with air and kept dry with cotton rolls which was placed into the buccal, labial vestibule and lingual pouch of the oral cavity. A filter strip was placed gently in the opening of the peri-implant margin 1 mm into the sulcus and left for 4 minutes. The fluid absorbed by the strip was eluted in 50µl Tris Buffer (50Mm Tris-Hcl, pH.5, 0.15M Nacl, 1Mm $Cacl_2$). The elute was then Centrifuged at 15000x and the supernatant was aliquoted and stored at -20 ^oC until assayed for MMP-8 using the Sandwich Enzyme Immunosorbent Assay (ELISA), Quantikine, Co, USA [25].

Peripheral blood extraction and serum collection: Five ml of peripheral blood was withdrawn under aseptic condition into dry pyrogen free tube. After centrifugation, the serum was pipetted from test tube and the following analyses were performed: Calcium levels were measured using the atomic spectrophotometry (Perkin Elmer Atomic Absorption Spectrophotometer) [26]. Osteocalcin was measured by ELISA [27] and alkaline phosphatase was measured as described by Varely [28]. TNFα quantitation was performed using the sandwich ELISA kit (Immuno Tech France) [29]. Serum Nitrate and Nitrite: it was determined in serum by Bories technique [30] and serum nitrite was quantitated calorimeterically after reaction with Geiss reagents.

Analysis of bone marker (serum calcium, osteocalcin and alkaline phosphatase), the level of nitric oxide (NO), TNFα and MMP-8 were performed for each subject immediately before implant placement, then after 1, 3 and 6 months after implant placement.

Statistical analysis:

Descriptive statistics as means and standard deviations were used. Student's t-test (unpaired) was performed for comparison between means before insertion of implant over-denture and the different follow up periods for both groups. Analysis of variance (ANOVA) with repeated measures to compare before insertion measurement and the subsequent follow up stages (1, 3 and 6 months) was performed. Bonferroni method was used to correct for type-I error. The Statistical Package for the Social Science software (version 16.0) was used for all analyses (SPSS, Chicago, IL, USA)

3. Results:

Table 1 showed a significant decrease in the level of MMP-8 over time in both groups, however, the decrease in the study group was more significant when compared to control group over time, (F=68.2), (*P*=0.001).

Table 2 showed a significant increase in the level of serum calcium over time in both groups, however, the increase in the study group was more significant when compared to control group at 3rd and 6th month, (P =0.002 and 0.001) respectively.
Table 3 showed a significant increase in the level of serum Osteocalcin over time in both groups, however, the increase in the study group was more significant (F=983.9) when compared to control group (F=1400.64). Serum osteocalcin level decreased in the control group at the 6th month.
Table 4 showed a significant increase in the level of serum alkaline phosphatase over time in both groups, however, the increase in the study group (F=53.07) was more significant when compared to control group (F=47.22).
Table 5 showed comparison between serum NO of the study group over time with the control group was not statistically significant. However, the serum nitrite of the study group was significant decreased more than the control group.
Table 6 showed a significant decrease in the level of TNFα in the 3rd and 6th month in study group. In contrast, there was a significant increase in the level of TNFα over time in the control group.

Table 1.Comparisons between MMP-8 levels in both groups .

Group	Time				F-Value
	Before Insertion	One month	3 months	6 months	
Study	123.0 (±14.0)	65.8 (±8.9)	50.0 (±7.9)	28.4 (±3.1)	68.2* (<0.001)
Control	122.0 (±14.4)	99.2 (±8.3)	77.6 (±2.1)	48.4 (±3.8)	76.3* (<0.001)
p-value	0.914	<0.001*	<0.001*	<0.001*	

p: *p* value for Student t-test between both groups at each period
F: ANOVA with repeated measures test with the adjusted Bonferroni was assessed in each group at the different months.
*: Statistically significant at $p \leq 0.05$

Table 2.Comparisons between Ca Levels (mg/dL) of both group

Group	Time				F-Value
	Before Insertion	One month	3 months	6 months	
Study	9.56 (±0.09)	9.85 (±0.08)	10.48 (±0.28)	10.67 (±0.23)	58.8* (<0.001)
Control	9.61 (±0.11)	9.76 (±0.06)	9.90 (±0.06)	9.92 (±0.04)	25.9* (<0.001)
p-value	0.547	0.079	0.002*	0.001*	

p: *p* value for Student t-test between both groups at each period
F: ANOVA with repeated measures test with the adjusted Bonferroni was assessed in each group at the different months.
*: Statistically significant at $p \leq 0.05$

Table 3.Comparisons between serum Osteocalcin Levels (mg/mL) of both groups

Group	Time				F-Value)
	Before Insertion	One month	3 months	6 months	
Study	6.29 (±0.20)	12.12 (±0.13)	22.20 (±0.84)	54.00 (±2.92)	983.92* (<0.001)
Control	6.34 (±0.15)	8.07 (±0.07)	8.27 (±0.12)	7.40 (±1.14)	1400.644* (<0.001)
p -value	0.666	<0.001*	<0.001*	<0.001*	

p: *p* value for Student t-test between both groups at each period
F: ANOVA with repeated measures test with the adjusted Bonferroni was assessed in each group at the different months.
*: Statistically significant at $p \leq 0.05$

Table 4. Comparisons between Alkaline Phosphate Levels (U/L) of both groups

Group	Time				F-Value
	Before Insertion	One month	3 months	6 months	
Study	45.2 (±3.9)	53.2 (±2.17)	61.0 (±2.92)	71.7 (±4.55)	53.072* (<0.001)
Control	42.28 (±1.84)	47.6 (±2.43)	49.4 (±1.14)	51.4 (±1.95)	47.227* (<0.001)
p -value	0.168	0.005*	<0.001*	<0.001*	

p: *p* value for Student t-test between both groups at each period
F: ANOVA with repeated measures test with the adjusted Bonferroni was assessed in each group at the different months.
*: Statistically significant at $p \leq 0.05$

Table 5. Comparisons between serum Nitrite Levels (Mmole/L) of both groups.

Group	Time				F-Value
	Before Insertion	One month	3 months	6 months	
Study	2.80 (±0.34)	8.82 (±0.54)	5.79 (±0.40)	4.04 (±0.09)	286.35* (<0.001)
Control	2.84 (±0.50)	8.82 (±2.91)	8.02 (±2.96)	7.24 (±2.87)	11.53* (0.001)
p-value	0.886	1.00	0.134	0.037	

p: *p* value for Student t-test between both groups at each period
F: ANOVA with repeated measures test with the adjusted Bonferroni was assessed in each group at the different months.
*: Statistically significant at $p \leq 0.05$

Table 6. Comparisons between TNFα Levels (Picogm/ml) of both groups.

Group	Time				F-Value
	Before Insertion	One month	3 months	6 months	
Study	177.4 (±7.92)	185.80 (±7.63)	156.60 (±4.98)	135.0 (±7.91)	261.64* (<0.001)
Control	177.0 (±7.91)	235.0 (±6.56)	277.0 (±7.91)	315.0 (±2.87)	303.23* (<0.001)
p-value	0.938	<0.001*	<0.001*	<0.001*	

p: *p* value for Student t-test between both groups at each period
F: ANOVA with repeated measures test with the adjusted Bonferroni was assessed in each group at the different months.
*: Statistically significant at $p \leq 0.05$

4.Discussion:

In contemporary dental prosthetic replacements, implant overdenture is considered the treatment of choice in completely edentulous patients to improve the oral function to achieve greater patient satisfaction. Great attention is given to overdenture therapy due to the increasing number of elderly patients seeking prosthetic dental replacements. Furthermore, this procedure is indicated in extremely atrophied ridges with poor bone quality that can not be treated using conventional fixed dental prosthesis [30].

The long-term stability of implant depends on their anchorage in bone. Thus, analysing markers of bone metabolism is essential tool to evaluate this. Therefore, serum calcium, osteocalcin and alkaline phosphatase were used in this study because they are considered as markers of bone formation Furthermore, these markers can be used to evaluate the effect of therapeutic agents to assess the efficacy of treatment and possibly reduce the need for the frequent bone density test [31].

TNFα and serum nitric oxide level were measured in this study because they are involved as important mediators for sepsis and play an essential role during the immune response [19,20]. MMP-8 level was used to evaluate the condition of peri-implant tissues; it was measured in this study in the peri-implant sulcus fluid because it is a useful specific and accurate marker to assess implant success [25].

The significant decrease of MMP-8 level in the study group could be attributed to the effect of oral administration of Ca-vit.D_3. Vitamin-D has a role in bone and calcium homeostasis, acts as an anti-inflammatory agent, inhibits immune cell cytokine expression and causes monocyte/ macrophages to secrete molecules that have a strong antibiotic effect. The reduction in the amount of MMP-8 level at 1st, 3rd and 6th months in the control group could be related to the active phase of bone and soft tissue remodelling that occurred due to implant placement [32,33].

The significant increase of serum calcium and osteocalcin level in the study group might be due to the effect of treatment with calcium and vitamin-D. The proper concentration of calcium ions is necessary for function of endocrine glands and the rigidity of bone. It controls the formation of mineralized tissue through regulation of the expression of various protein in these cell notably matrix collagenous and non-collagenous species, enzymes, and calcioproteins. Ca-Vit.D enhance soft tissue healing and bone maturation.[1] The serum alkaline phosphatase was significantly increased in the study group as it was considered as an indication of osteoblastic activity, marker of bone turnover and plays a role in osteoid formation and mineralization. This increase could be attributed to the oral administration Ca- Vit.D that induces the

formation of a layer of distinct calcified tissue over the surface of the residual bone. The systemic condition created by Ca-Vit. D deficiency influences the remodelling process of alveolar ridge and leads to change of size and shape of ridge [14,31].

The significant decrease of nitric oxide, observed in the study group, could also be attributed to calcium and vitamin-D which minimized the production of nitric acid at the implant site and significantly attenuated the pathogenesis of acute rejection, resulting in prolonged survival of the implant. In addition, a significant reduction in the cellular infiltrate induced by inhibition of nitric oxide production suggest a potential role for nitric oxide in the emigration of inflammatory cells into organ undergoing rejection [16,34].

The significant elevation of concentration of nitric oxide in the control group might be due to the production of the free radical nitric oxide during implant placement that resulted in depression of immune response as a result of cytokines that initiated macrophages and induced immune-incompetence [35].

The production of TNFα in the control group was higher than the study group because TNFα is a central mediator of acute inflammation released rapidly or secreted at the site of injury or infection. TNFα is a pluripotent cytokines, which appear to play a central role in inflammatory disease, and possesses bone resorptive properties. However, the lowest production of TNFα in study group may be due to the effect of calcium and vitamin-D. TNFα mediate the proliferation of fibroblasts and endothelial cells, which are important elements of wound healing around dental implant, while excessive production of TNFα could lead to tissue damage. Production of TNFα is usually inhibited by the presence of cyclosporine A, prostaglandin E2 and dexamethasone [17,36]. One limitation to this study is that the number of participants was only 10 subjects. The reason to that was the stringent inclusion criteria used since it was difficult to recruit healthy edentulous subjects with no systemic disease such as diabetes and/or hypertension that may affect the results.

4. Conclusion:

Within the limitation of the study, it could be concluded that the identification and management of nutritional deficiencies is a useful adjunct to successful implant treatment. Improvement of the systemic condition of the patient is necessary to enhance alveolar bone formation. Vitamin-D and calcium supplements could be beneficial in arresting or slowing the progression of bone loss and improving the immunity. The preliminary results of this study warrant further investigations with random clinical trials using larger samples.

Conflict of Interest

The authors of the manuscript do not have any direct financial relation with the commercial identities mentioned in the paper that might lead to a conflict of interest for any of the authors. All work was done at King Abdulaziz University, Faculty of Dentistry, Jeddah, Saudi Arabia and was approved by Research Ethical committee, King Abdulaziz University, Faculty of Dentistry.

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Corresponding author:
Dept. of Oral and Maxillofacial Rehabilitation.
King Abdulaziz University. Removable division,
Dental Hospital. P.O.Box 80209
Jeddah, 21589 Kingdom of Saudi Arabia Email: alkhiary@gmail.com

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