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Advances in Bioresearch

## ORIGINAL ARTICLE

# Resonance frequency analysis of dental implant stability by determination of bone metabolic marker levels in peri-implant crevicular fluid between diabetic and non-diabetic patients

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**ABSTRACT**
*The aim of the study was the early prediction of the stability of implants in diabetic and nondiabetic subjects by correlating with the study of biomarker assay such as alkaline phosphatase (ALP) and receptor activator of nuclear factor kappa B ligand (RANKL) in the peri-implant crevicular fluid prior to the prosthetic loading of the implants. This was a prospective study of 20 patients, 10 patients with a history of diabetes mellitus (HbA1C > 6%) and under medication) and 10 patients clinically healthy, with single missing mandibular posterior teeth. The patients received endosteal root form implants. All the patients were subjected to peri-implant crevicular fluid collection and resonance frequency analysis for implant stability measurements on day 1, day 30 and day 90 post implant placement. This study showed that all the implants placed in the diabetic group and nondiabetic group attained stability and successful osseointegration as indicated by implant stability quotient (ISQ). This finding could possibly be correlated with the significantly lower levels of ALP and significantly higher levels of RANKL found in the peri-implant crevicular fluid of the diabetic group. The results of our study have further confirmed that diabetes mellitus with good to average glycemic control is not an absolute contraindication for dental implant placement, but the implants placed in the diabetic group required more critical monitoring and longer duration to complete the healing cascade. More importantly, maintaining the blood sugar levels under control during the post placement healing period is vital for the success of the procedure.*
***Key words:*** *Diabetes, implant stability, radiofrequency analysis, biomarker assay*

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## INTRODUCTION

Functional and esthetic rehabilitation of partially and completely edentulous patients with the use of dental implants has become the most popular and successful treatment modality in modern dentistry. These implants have largely evolved over time with many developments in the materials and the form of implants. Despite these advances, the most important and deciding factor for the success or failure of an implant is osseointegration. [1,2,3]

According to Branemark, osseointegration is known as the structural contact between the native bone component and the surface of the implant at a histological level.[4] Clinically, this is evaluated as the absence of any movement or mobility between the implant surface and alveolar bone when the implant is subjected to functional loading after completion of the healing cascade.

Osseointegration of implants involves a series of cellular and molecular events that occur at the bone to implant interface and over the surface of the implant, finally effecting in formation of new bone providing the implant with adequate stability indicating complete healing of the implant.

The degree of osseointegration achieved by the implant in the post-implant insertion time-period defines the long-term success or failure of the implant placed which so far had been based on only clinical

assessment and imaging which is arbitrary. A definitive prognostic indicator with biochemical assay of peri implant crevicular fluid could furnish the prognosis of implant uptake.

Diabetes mellitus is a commonly occurring metabolic disorder caused owing to hyperglycemia or increased blood sugar levels. Uncontrolled diabetes in the long term makes a patient susceptible to multiple complications caused by micro- and macroangiopathies. This also raises the risk for gingivitis and periodontitis.

Any damage to the periodontium carries an equal risk of failure of implant healing due to inadequate bone formation and osseointegration. Owing to these factors, there is a general myth that success of implants is low in patients with diabetes mellitus. Periodic monitoring of the stability and healing of implants is very important in diabetic patients on a clinical and molecular level. At the molecular level, healing is studied by indirect indicators known as biomarkers present in the gingival crevicular fluid surrounding the implant, the levels of which will predict the degree of osseointegration that has occurred.

It was found that greater activity of alkaline phosphatase was present in the areas of tension compared to areas of compression. These results inferred that the levels of alkaline phosphatase in the gingival crevicular fluid indirectly reflects the biologic activity in the periodontium during the process of orthodontic tooth movement [9]

Receptor activator of nuclear factor kappa b (RANK) is a prominent cytokine reflecting the process of osteoclastogenesis by its process of binding directly to the RANK receptor on the surface of the preosteoclasts and osteoclasts which causes both the differentiation of osteoclast progenitors and promotes the activity of mature osteoclasts. [7].

The present study hypothesized that an evaluation of multivariate parameters such as clinical stability analysis and biochemical markers around dental implants will contribute to better assessment of the implant success especially in patients with systemic diseases like diabetes and osteoporosis.

The objective of the study was the early prediction of the stability of implants in diabetic and non-diabetic subjects by correlating the levels of biomarker assays such as alkaline phosphatase (ALP) and receptor activator of nuclear factor kappa B ligand (RANKL) in the peri-implant crevicular fluid.

## MATERIAL AND METHODS

This prospective, clinical, randomized control study was carried out in the Department of Oral and Maxillofacial Surgery between April 2017 and November 2018 in patients with single missing tooth requiring rehabilitation with Dental Implant placement. The protocol for this study was reviewed, discussed, and approved by the Institutional Review Board of our Institution with protocol number MADC/IRB-XIV/2017/263.

The study sample included 20 patients of age ranging from 23 to 65 years. All the patients had previously undergone extraction of the mandibular molar and necessitated restoration of tooth form and function by placement of dental implant after complete healing and ossification of the extraction socket as evidenced by radiographs.

Ten patients had no history of diabetes mellitus or glycosylated hemoglobin levels less than 6 % and ten patients had a history of diabetes mellitus or glycosylated hemoglobin levels more than 6 % but kept under control with hypoglycemic agents.

Pre-operative panoramic radiographs and cone-beam computerized tomograms (CBCT) were obtained for all the patients based on which the dimensions of the implants were decided.

Post operative radiographs were taken to assess the degree of osseointegration that had occurred. The implant stability was also assessed using resonance frequency analysis and biomarker assay of peri-implant crevicular fluid for 3 months following the placement of the implants.

The surgical procedure was performed under local anesthesia with 2% lignocaine (with 1:80,000 adrenaline), crestal incision was placed in the posterior mandibular edentulous area. Mucoperiosteal flap was elevated to expose the osteotomy site. The osteotomy site was then marked with a pilot drill following which sequential enlargement of the osteotomy site was done in accordance with the planned implant dimensions. Following this, the implant was driven into the prepared osteotomy site to achieve an initial torque of 35-40 N-cm. A gingival former was secured onto the implant in all the cases to achieve adequate gingival cuff formation around the implant. The flap was then repositioned and secured with sutures. [Fig 1] All patients were prescribed antibiotics and analgesics for 5 days post implant placement. Patients are also asked to regularly use chlorhexidine mouth wash.

Following adequate isolation and drying of the surgical site, sterile 0.4% taper adsorbent paper points were gently inserted into the crevice of implants and left in place for 30 seconds. Strips contaminated with blood were discarded. Samples were obtained from buccal and lingual aspects of implants [Fig 2]. After peri-implant crevicular fluid collection, strips were placed in Eppendorf tubes and preserved in 20µl phosphorous buffered saline and stored at -30 degrees Celsius before laboratory analysis which was carried out within 24 hours of sample collection for the respective biomarkers. Peri-implant crevicular fluid collection was done on the day of implant placement, and post-placement days 30 and 90 respectively, prior to cementation of the prosthetic crown.

The samples were then subjected to enzyme-linked immunosorbent assay (ELISA) for evaluation of levels of receptor activator of nuclear factor kappa B ligand (RANKL) analysis and liquid chromatography-mass spectrometry for evaluation of levels of alkaline phosphatase (ALP).

Resonance frequency analysis for implant stability measurement as an indirect indicator of osseointegration is carried out using Osstell™ Mentor ISQ (Integration Diagnostics AB, Goteborg, Sweden) instrument by inserting a standardized abutment smart-peg attachment of fixed length into each implant. The transducer mentor probe is held at a distance of 2-3 mm from the magnetic tip of the smart-peg till the instrument beeped and displayed the ISQ reading. [Fig 3]

Resonance frequency analysis for implant stability was carried out with the Osstell™ Mentor device on day 0 and post-implant placement days 30 and 90 respectively. [Figures 4,5,6]

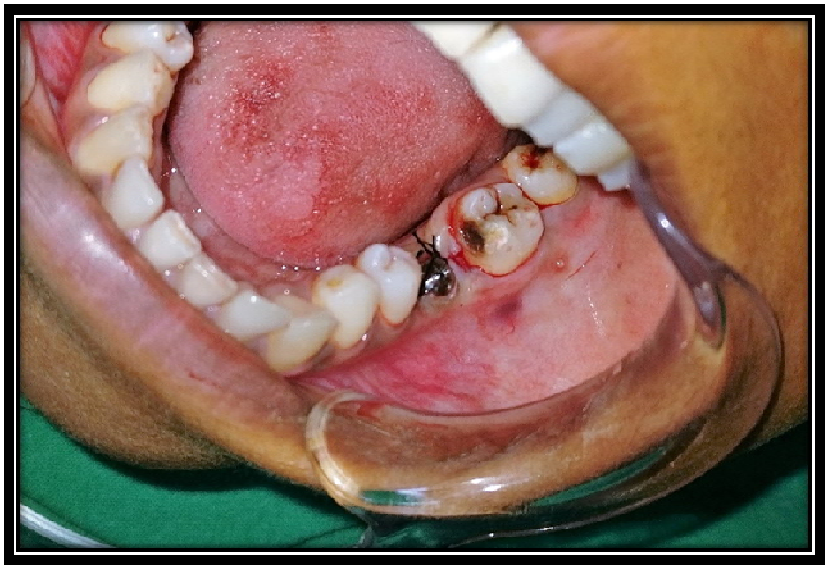


**Figure 1:** Day 0 - Implant placement

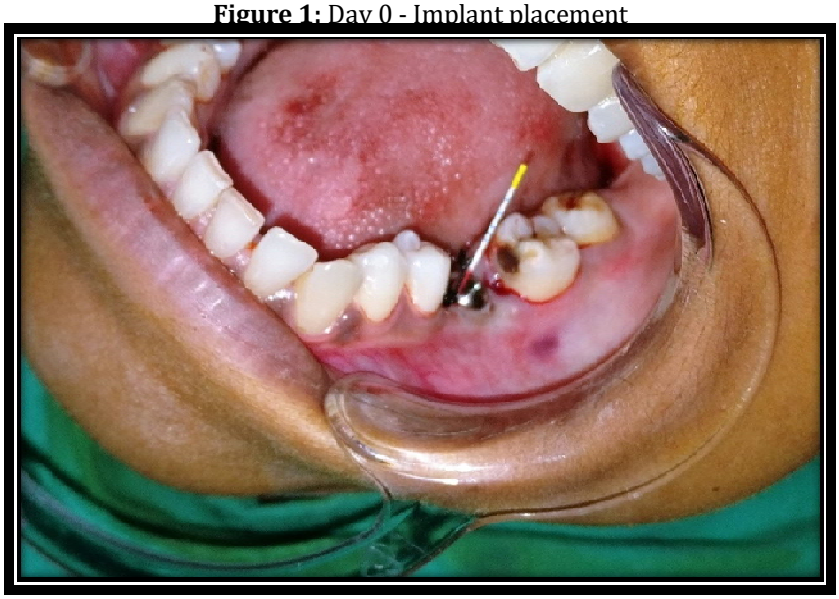


**Figure 2:** Collection of peri-implant crevicular fluid

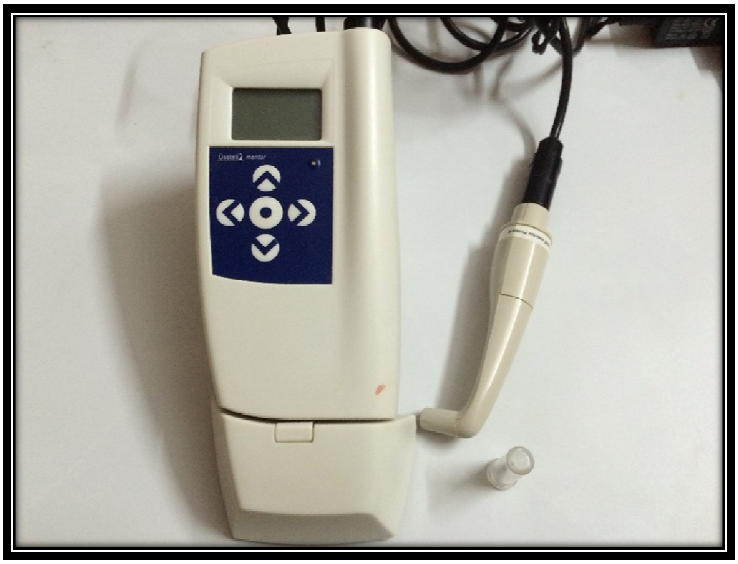


**Figure 3**: Osstell Mentor device


**Figure 4**: Implant stability measurement on day 0

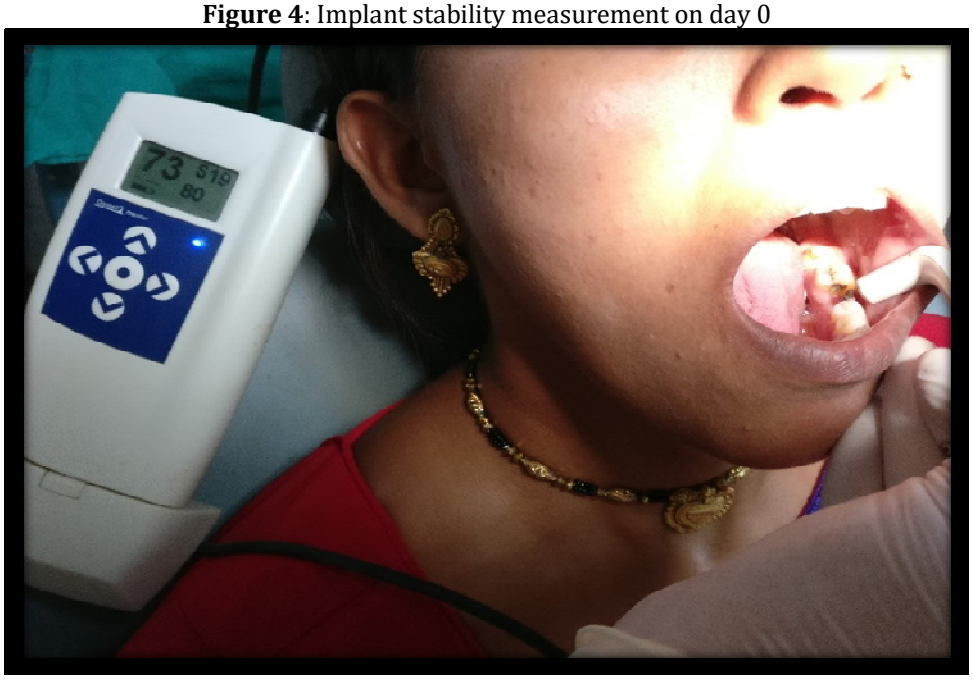



**Figure 5:** Implant stability measurement on day 30

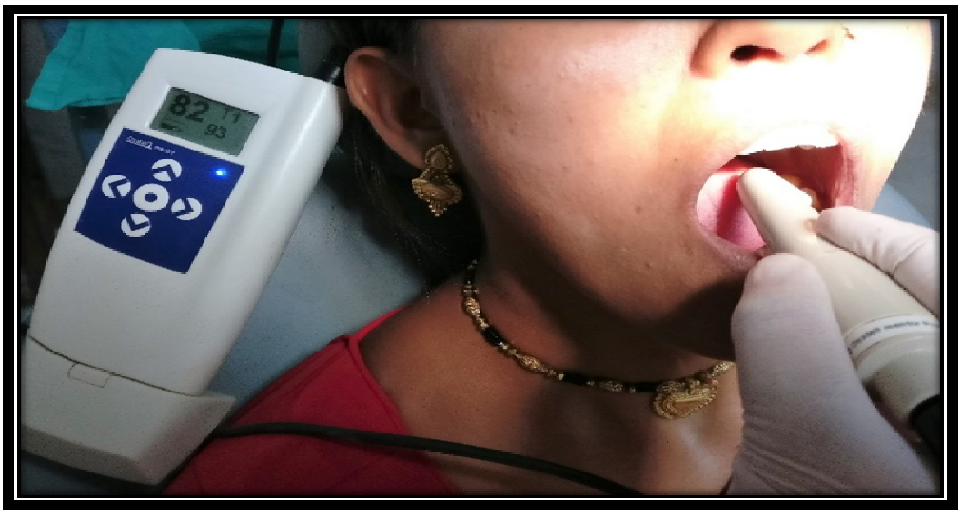


**Figure 6:** Implant stability measurement on day 90

**RESULTS**

The implant stability values (ISQ) and levels of biochemical markers (alkaline phosphatase and RANKL) present in the peri-implant crevicular fluid were collected and analyzed, following which the values obtained on the day of implant placement and post-placement days 30 and 90 were tabulated. The significance of intergroup difference for the outcome variables were evaluated using a t-test for equality of means by comparing day 1, day 30, and day 90 values. The significance of intra group relation for the recorded data were evaluated using chi-square test.

The changes in the levels of RANKL and Alkaline phosphatase along with radiofrequency analysis, a function of implant stability, were statistically analyzed and compared using a t-test n the diabetic and non-diabetic groups.

**Alkaline phosphatase:**

Levels of alkaline phosphatase were found to be statistically significant ($p<0.05$) for day 30 and day 90 ($p<0.05$) follow-up between the diabetic and non-diabetic group with the levels of alkaline phosphatase significantly decreasing for the diabetic group in comparison to the non-diabetic group indicating decreased bone mineralization around the implant. **[**Tables 1, 2]

**RANKL:**

Measurements of the levels of RANKL were statistically significant ($P<0.05$) for Day 30 and day 90 ($P<0.05$) follow ups between the diabetic and non-diabetic group with the levels of RANKL significantly increasing for the Diabetic group in comparison to the non-diabetic group indication increased bone resorption around the implant. [Tables 3, 4]

**Radiofrequency analysis (RFA):**

The results yielded were found to be statistically significant ($p<0.05$) for resonance frequency analysis of day 90 between the diabetic and non-diabetic group with the stability of the diabetic group being significantly less than the non-diabetic group. **[**Tables 5, 6]

**Table 1:** Mean levels of Alkaline phosphatase between the study groups with statistical analysis of significance

| | GROUP | N | Mean (U/l) | P value |
|---|---|---|---|---|
| **ALP - DAY1** | NON DIABETIC | 10 | 70.26 +/- 13.59 | 0.136 |
| | DIABETIC | 10 | 61.07 +/- 12.76 | |
| **ALP - DAY 30** | NON DIABETIC | 10 | 89.58 +/- 8.87 | |
| | DIABETIC | 10 | 57.75 +/- 10.17 | **0.000** |
| **ALP - DAY 90** | NON DIABETIC | 10 | 113.70 +/- 19.11 | |
| | DIABETIC | 10 | 74.77 +/- 10.27 | **0.000** |

**Table 2**: Error bar diagram depicting mean Alkaline phosphatase levels between the study groups

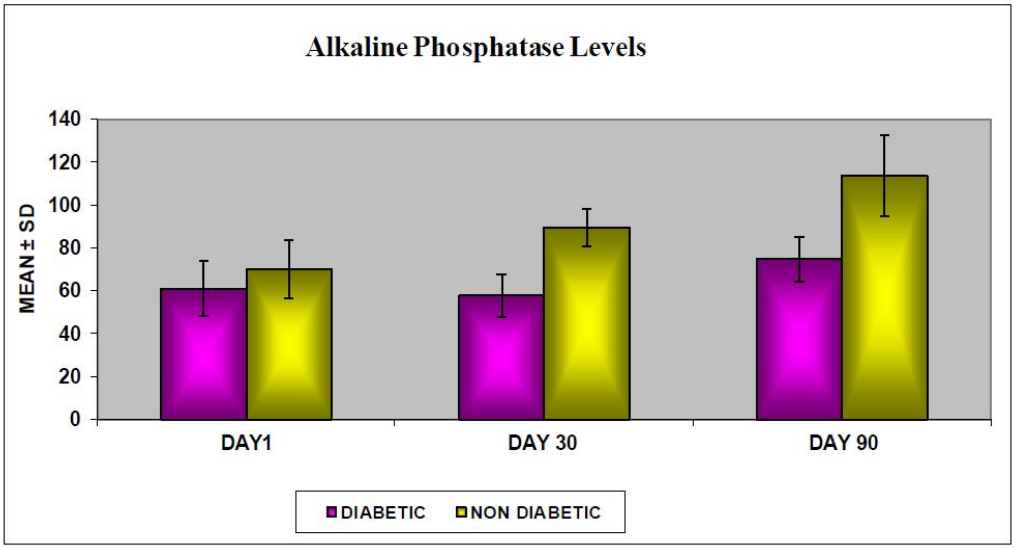



**Table 3:** Mean levels of RANKL between the study groups with statistical analysis of significance

| | GROUP | N | Mean (ng/L) | P value |
|---|---|---|---|---|
| **RANKL - DAY1** | NON DIABETIC | 10 | 47.85 +/- 6.38 | 0.104 |
| | DIABETIC | 10 | 42.99 +/- 6.29 | |
| **RANKL- DAY 30** | NON DIABETIC | 10 | 44.34 +/- 3.52 | |
| | DIABETIC | 10 | 54.68 +/- 5.66 | **0.000** |
| **RANKL - DAY 90** | NON DIABETIC | 10 | 35.05 +/- 4.79 | |
| | DIABETIC | 10 | 40.77 +/- 4.77 | **0.015** |

**Table 4:** Error bar diagram depicting mean RANKL levels between the study groups

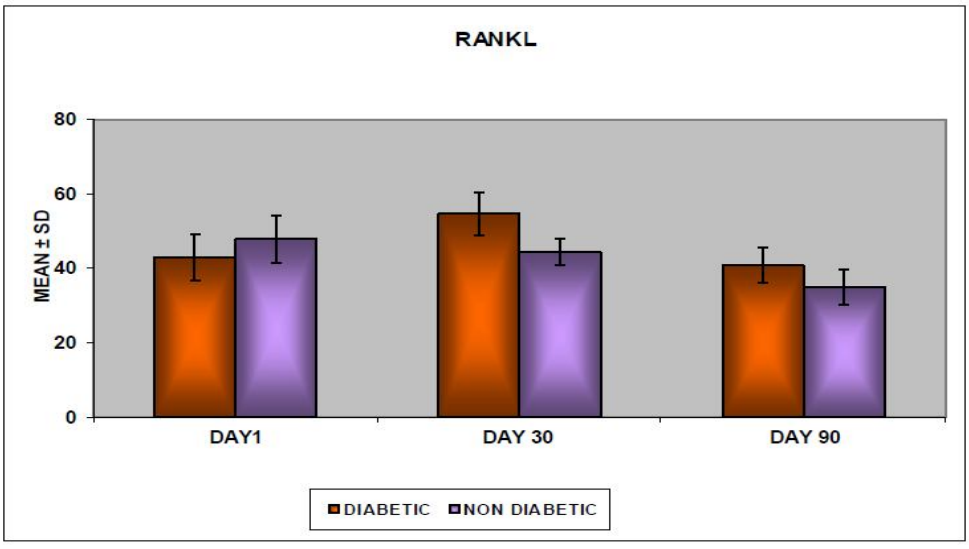
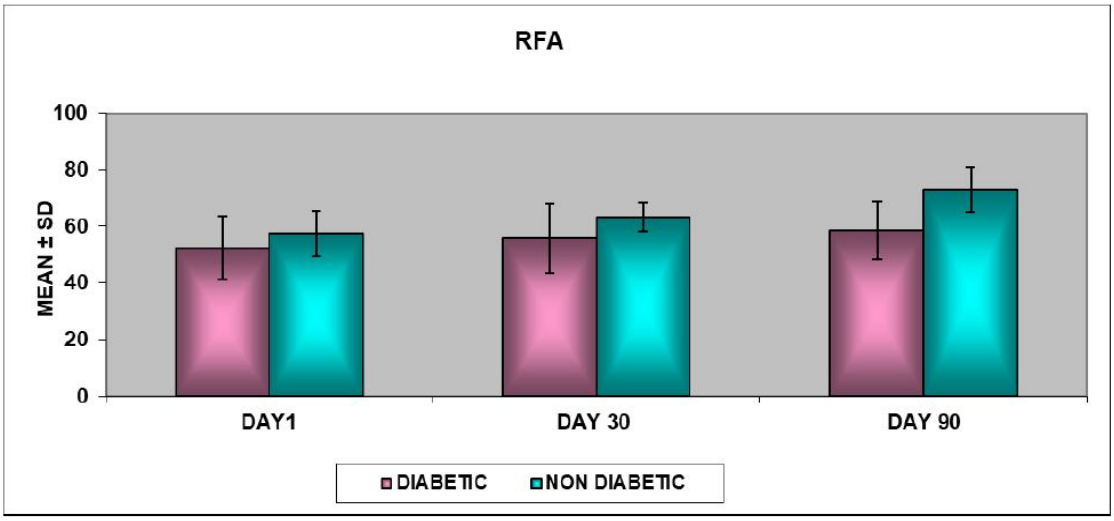


**Table 5:** Mean RFA values between the study groups with statistical analysis of significance

| | GROUP | N | Mean | P value |
|---|---|---|---|---|
| RFA - DAY1 | NON DIABETIC | 10 | 57.30 +/- 8.097 | 0.275 |
| | DIABETIC | 10 | 52.40+/- 11.11 | |
| RFA - DAY 30 | NON DIABETIC | 10 | 63.30 +/- 5.23 | |
| | DIABETIC | 10 | 55.80 +/- 12.15 | 0.090 |
| RFA - DAY 90 | NON DIABETIC | 10 | 72.90 +/- 7.97 | |
| | DIABETIC | 10 | 58.60 +/- 10.16 | **0.003** |

**Table 6:** Error bar diagram depicting mean RFA values between the study groups




## DISCUSSION

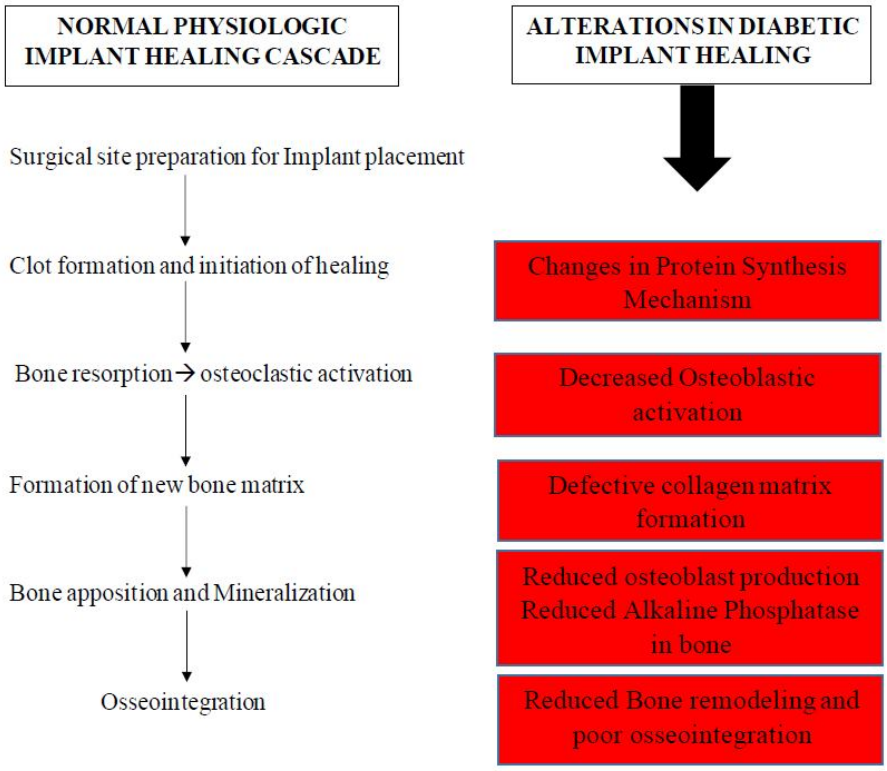
The purpose of this study was to evaluate the degree of osseointegration achieved in patients with diabetic mellitus by indirect measures of biomarkers and implant stability. Osseointegration was defined by Branemark as "a direct structural and functional connection between ordered living bone and the surface of a load-carrying implant" [4].

However, the degree of osseointegration is influenced by multiple systemic and local factors which are responsible for the successful healing of implants if conducive or for the failure of the implant if even one of the factors fail irreversibly. One of the main causes for risk of poor implant healing or a disruption in the normal healing is a chronic, metabolic disorder due to hyperglycemia known as diabetes mellitus. Poor glycemic control and hyperglycemia have been directly associated with increased susceptibility to peri-implantitis, impaired osseous wound healing, with an adverse effect on several stages of the bone healing process. It also produces a deleterious effect on the bone matrix and its components affecting the adherence, growth and accumulation of extra-cellular matrix **[Fig 7].** Diabetes also has an inhibitory effect on the mineral homeostasis, production of osteoid and finally bone formation. [5]

According to Mellado-Valero et al [6] in patients with diabetes, a reduced level of bone-implant interface contact was present, which was reversible by treatment with appropriate hypoglycemic agent and lifestyle modifications. The critical time frame for monitoring was the first year, post implant placement subject to functional loading as most implants were seen to fail during this period in the diabetic population. Compared with the non-diabetic population, a higher failure rate was seen in diabetic patients, possibly due to the microvascular complications of diabetes mellitus.

**Figure 7:** Normal implant bone healing versus altered healing cascade in diabetic patients




Javed and Romanos (2009) [5] conducted a meta-analysis and systematic review on the influence of increased blood sugar levels on the process of osseointegration and found that when the blood glucose levels were kept under optimal control, implant osseointegration does occurred successfully.

Gingival crevicular fluid is the thin film of inflammatory exudate that is present at the gingival margin or within the gingival crevice. The components of this fluid were analyzed for the release of factors responsible for host response mechanism which serve as biomarkers or indicators of good periodontal health or the occurrence of a destructive process. [7]

Alkaline phosphatase is a calcium and phosphate binding protein and a phosphorhydrolytic enzyme. Bone-specific alkaline phosphatase (BALP) is an important indicator of bone formation by its phenotypic expression in the bone forming osteoblastic cell and is also known to be involved in bone calcification and mineralization process. The normal levels of alkaline phosphate in the crevicular fluid ranges from 40-140 U/l. The activation of alkaline phosphatase was found to play a very important role in the bone mineralization process involving matrix vesicles, making this an effective marker of osteoinduction or bone formation process. [8,9]

Receptor activator of nuclear factor kappa B ligand is a type II membrane protein of the Tumor necrosis factor (TNF) superfamily.[7] It binds to RANK receptor in the cells of myeloid lineage like the osteoclasts and promotes the differentiation and activation of the osteoclasts effecting in bone resorption.

In our study there was significant difference ($p<.0001$) in ALP values between the non-diabetic and the diabetic groups on day 30 and 90. [Table 1]. The increased levels of ALP in the non-diabetic group, was a function of increased bone formation and deposition around the implant.

There was also a significant difference in RANKL values between the non-diabetic and the diabetic groups on day 30 ($p<.0001$) and day 90 ($p<.05$). [Table 3]. The increased levels of RANKL in the diabetic group, was an indicator of increased osteoclastic activity in the diabetic group.

Dursun and Tozum (2016) [10] conducted a meta-analysis to systematically review the biomarkers and enzymes associated with various manifestations of peri-implant disease process and the influence of these on the pathogenesis of the inflammatory diseases around dental implants. Among all studies reviewed, the levels of matrix metalloproteinases were found to be positively correlated with the clinical signs of inflammation around the implants. The levels of interleukins and tumour necrosis factors were the major indicators of bone remodeling. The levels of RANKL and osteoprotegerin were found to be significantly higher in the sites of peri-implantitis around the implant. **[**Tables 7,8]

**Table 7:** BONE FORMATION MARKERS

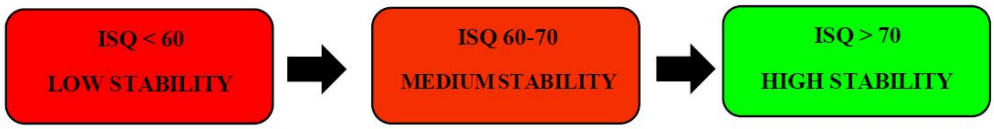
| |
|---|
| Bone Alkaline Phosohatase (ALP) |
| Osteocalcin (OC) |
| C-terminal propeptide type I procollagen (PICP) |
| N-terminal propeptide of type I procollagen (PINP) |
| Collagenase-3 / Matrix metalloproteinase -13 |
| Osteonectin |
| Osteopontin (OPN) |

**Table 8:** BONE RESORPTION MARKERS

| |
|---|
| Hydroxyproline, total and dialyzable (OH-Pro,OHP) |
| Receptor activator of nuclear factor kappa B ligand (RANKL) |
| Pyridinoline (PYP, Pyr) |
| **Deoxypyrindoline (DPD,d-Pyr)** |
| **Cross-linked C-terminal of type I collagen(ICTP)** |
| **Cross-linked C-termianltelopeptide of type I collagen (fragments alpha-CTX, beta-CTX)** |
| **Cross-linked N-terminal telopeptide of type I collagen (fragments NTX)** |
| **Hydroxylysine-glycosides (Hyl-Glyc)** |
| **Bone sialoprotein (BSP)** |
| **Tartarat-resistant acid phosphatase (TR-ACP)** |
| **Free gamma carboxyglutaminacid (GLA)** |
| **Cathepsin B** |
| **Collagenase -2 / Matrix metalloproteinase 8** |
| **Gelatinase / Matrix metalloproteinase 9** |
| **Interleukin -8** |
| **Calprotectin** |

While biochemical marker evaluation gives information regarding the osseointegration at a more molecular level involving laboratory estimation for the levels of the biomarkers, another aspect of evaluation of the healing is by analysis of the stability of the implant which is a more clinical indication of the healing, this can be performed by an indirect method of measuring the Implant stability quotient (ISQ) using the Osstell Mentor resonance frequency analysis. Resonance frequency analysis stability measurements basically applies a vibrational theory to measure stiffness of the implant by applying a false bending load or a shear force which mimics the clinical load that an implant is normally subjected to and ultimately provides indirect information about the rigidity of the implant–bone junction. [2] **[Fig 8]**
The basic principle of resonance frequency analysis stability measurements is that it essentially applies a bending load, which mimics the original clinical load an implant is subjected to and provides hence provides information about the rigidity of the implant–bone junction. Resonance frequency analysis technique can provide relevant information about the state of the implant–bone interface at any stage of the treatment or at follow-up examinations. It has been shown that implants with high ISQ values during regular follow-up checking are estimated to have successfully osseointegrated, while low and decreasing ISQ values may be a sign of implant failure and / or marginal bone loss which will permit early intervention. [11]

**Figure 8**: Significance of implant stability quotient values as displayed through the Osstell mentor



Han et al [12] performed a similar study where the levels of Osteoprotegerin (OPG) and RANKL were evaluated in the peri implant crevicular fluid and compared with implant stability quotient in a group of 35 healthy patients with follow-up till 12 weeks post insertion. It was concluded that the level of OPG raised progressively while levels of RANKL was lesser than OPG levels 12 weeks following implant healing. The implant stability increased steadily from the day of implant placement till 12 weeks post placement. In our study as well, the implant stability increased progressively in both study-groups, but the increase in the implant stability quotient (ISQ) was more significant in the non-diabetic group.
In our study there was significant difference ($p<.005$) in RFA ISQ values between the non-diabetic and the diabetic groups at day 90 [Table 5]. The non-diabetic group had an average value of 72.9 (ISQ > 70) at day 90, which is considered high stability for an implant. The diabetic group had an average ISQ value of 58.60, just under the cut-off value of 60, which is considered low stability for an implant. This finding could possibly be correlated with the significantly lower levels of ALP and significantly higher levels of RANKL found in the peri-implant crevicular fluid of the diabetic group.
However, this study showed that implants placed in the diabetic group also attained stability and successful healing, but these implants required more critical monitoring and required longer duration to complete the healing cascade. More importantly, maintaining the blood sugar levels under control during the post placement healing period is also for the success of the procedure.

## CONCLUSION

This study of biomarker assay in the peri implant crevicular fluid gives a scientific guideline to the predictability of implant uptake in diabetic and non-diabetic patients. The results of our study have further confirmed that diabetes mellitus with good to average glycemic control is not an absolute contraindication for dental implant placement. The rate of healing is prolonged in patients with diabetes compared to non-diabetic patients which necessitates delayed loading of the implants with the prosthetic component.

## Funding

No funding was received for this study.

## Conflict of Interest/ Competing interests

The authors declare that they have no conflict of interest.

## Ethical approval

This article does not contain any studies with animals performed by any of the authors.

## Ethical approval

"All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

## Informed consent

Informed consent was obtained from all individual participants included in the study.

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