

To Determine Correlation between Resonance Frequency Analysis and Alkaline Phosphatase Level during the Healing Period of Dental Implant

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Abstract

Background: The aim of the present study is to determine correlation between resonance frequency analysis (RFA) and alkaline phosphatase (ALP) level during the healing period of dental implants. **Materials and Methods:** This study was performed during 3-month healing period of implant placement. RFA assessment along with gingival crevicular fluid (GCF) and peri-implant crevicular fluid (PICF) collection from the implant site and contralateral tooth was done before, at the time, and 1st, 4th, 8th, and 12th week of implant placement from 20 patients. ALP levels were measured using colorimetric analysis. The collected data were statistically analyzed using the Pearson correlation coefficient and paired *t*-test. **Results:** The mean GCF values of 8th and 12th week were highly significant with $P < 0.001$. The mean PICF values of the 1st, 4th, 8th, and 12th week were highly significant with $P < 0.001$. RFA was assessed at the day of implant placement, 1st week, 4th week, 8th week, and 12th week post-implant insertion the mean values of 1st, 8th, and 12th week post-implant placement were highly significant with $P < 0.001$. The ALP levels in GCF and PICF in correlation with RFA were statistically non-significant. **Conclusion:** From the collected data, it can be inferred that these two entities are totally independent of each other and can be used as a valuable tool in measuring implant stability individually at the time of implant placement and during the healing period of implants.

Keywords: Alkaline phosphatase, Biomarker, Gingival crevicular fluid, Implant stability quotient, Osteocalcin, Peri-implant crevicular fluid, Resonance frequency analysis.

INTRODUCTION

The aim of any replacement therapy is to achieve esthetics and function with a maximum success rate. Dental implants have shown a high success rate for the rehabilitation of patients if certain conditions are met during and after the treatment.^[1] The primary requirement of dental implants is a biologic process called “osseointegration.” Thus, the implant and the bone itself in which it has to be integrated are of primary concern. The third parameter which plays an important role is the mechanical and chemical mediation of osseointegration.^[2]

Achieving and maintaining implant stability are prerequisites for successful clinical outcome. Implant stability is achieved at two different stages: Primary and secondary. Primary stability comes from mechanical engagement with the cortical bone. It is affected by the quantity and quality of bone that the implant is inserted into, surgical procedure, length, diameter, and form of the implant.^[3] During healing, this primary stability is replaced by a biological bonding of newly

formed bone to the implant surface and is termed as secondary stability. The time of functional loading is dependent on the secondary stability.^[4]

The measurement of implant stability is based on clinical, histological, biomechanical, and biochemical parameters.^[5] Some of the methods for analyzing dental implant stability are histological analysis, percussion test, radiographs, reverse torque, cutting resistance, and resonance frequency analysis (RFA).^[3] The RFA technique gives the clinician the possibility to measure implant stability at various times during treatment. Hence, the anchorage of the implant to bone can be

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mechanically evaluated and assessed at the time of insertion, healing, and loading of the implant.^[3]

A sequence of immune-inflammatory responses followed by angiogenesis and eventually osteogenesis takes place over a period of 8–12 weeks. The lamellar bone initiates biological stability.^[6] This complex and dynamic process may occur through contact osteogenesis, where the implant surface is populated by bone cells after fixation to form *de novo* bone, or through distance osteogenesis, where bone formation is preceded by the osteoclastogenesis of the existing tissue.

Several hormones involved are parathyroid, vitamin D, growth hormone, steroids, calcitonin, and various medullary cytokines. Growth factors such as M-CSF, receptor activator of NF- κ B ligand (RANKL), VEGF, and the interleukin 6 family are importantly involved as well. The osteoclasts-osteoblast-immune cell-cytokine complex is known as the basic multicellular unit (BMU). The duration for a complete remodeling of the BMU is the bone remodeling period.^[7]

The structures that are most probably involved in the earliest phases of calcification are collagen fibers, matrix vesicles, and crystal ghosts. Alkaline phosphatase (ALP) is released in the gingival crevicular fluid (GCF) by the polymorphonuclear neutrophils during inflammation, osteoblast during bone formation, and periodontal ligament fibroblasts during periodontal regeneration.^[8] The activation of ALP plays a very important role in the mineralization process involving matrix vesicles.

ALP can be easily collected from GCF and peri-implant crevicular fluid (PICF) for enzyme analysis. Thus, the evaluation of PICF, a non-invasive, clinical, and biochemical approach can be used to assess and predict the amount of bone formation around the implant.

Both mechanical and chemical changes go hand in hand during the insertion and healing period of implants, all the aids of the loading of prosthesis. Thus, a need arises to determine and correlate the relation evaluation are of utmost importance for between RFA and ALP biomarkers at the time of implant insertion and during the healing period.

MATERIALS AND METHODS

This “*in vivo*” study was designed and conducted to monitor the primary and secondary stability of dental implants during 3-month healing period for osseointegration using RFA; to monitor the levels of ALP during the healing period of 3 months; and to find out correlation between ALP levels and RFA values. Twenty patients were selected randomly from the OPD in the department of prosthodontics and a written consent form was obtained which was duly signed by patients before making them the part of research.

Inclusion criteria

The following criteria were included in the study:

1. Partially edentulous patients belonging to the age group of 25–65 years were included in the study

2. Patients with adequate bone volume were included in the study
3. Patients with adequate oral hygiene were included in the study
4. Patients with healthy contralateral teeth were included in the study
5. Patients with no systemic diseases (hypothyroidism, hyperthyroidism, osteoporosis, etc.) were made a part of the study
6. Non-smoker patients were made part of the study
7. Patients having no history of radiotherapy of the head and neck region were made a part of the study.

Exclusion criteria

The following criteria were excluded from the study:

1. Patients presenting any periodontal or periapical lesion were excluded from the study
2. Postmenopausal women were not a part of the study
3. Pregnant and lactating females were excluded from the study
4. Patients with a history of any periodontal therapy in the past 3 months were not made part of the study
5. Patients suffering from any systemic disease disrupting the levels of ALP were excluded from the study
6. Patients with the need for submersion of implants were excluded from the study
7. Patients presenting with the requirement of bone augmentation at the implant site were not made a part of the study.

GCF collection from the contralateral natural tooth

GCF was collected by placing the microcapillary pipette in the gingival sulcus of the contralateral tooth before the surgical procedure and after the 1st, 4th, 8th, and 12th week. It was immediately transferred to airtight plastic vials containing 1.5 uL of phosphate buffer, and further, biochemical analysis was performed.

Surgical placement of implant at the site

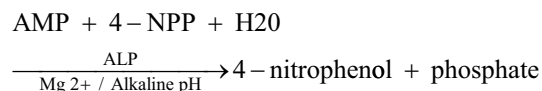
After clinical, hematological, and radiographic examination, endo-osseous implant was placed at specified sites. Complete isolation of the implant collar was achieved and smart peg was fixed to the internal hex of the implant head, using RFA primary stability at the time of implant insertion was calibrated, and a smooth polished gingival former was screwed to the implant connection. The second sample of GCF and the first sample of PICF were collected after adequate isolation.

The patients were recalled after 7 days for suture removal and the sample of GCF and PICF for 1st week were also taken along with RFA assessment. The samples were periodically collected during recall at the 1st, 4th, 8th, and 12th week of the healing period.

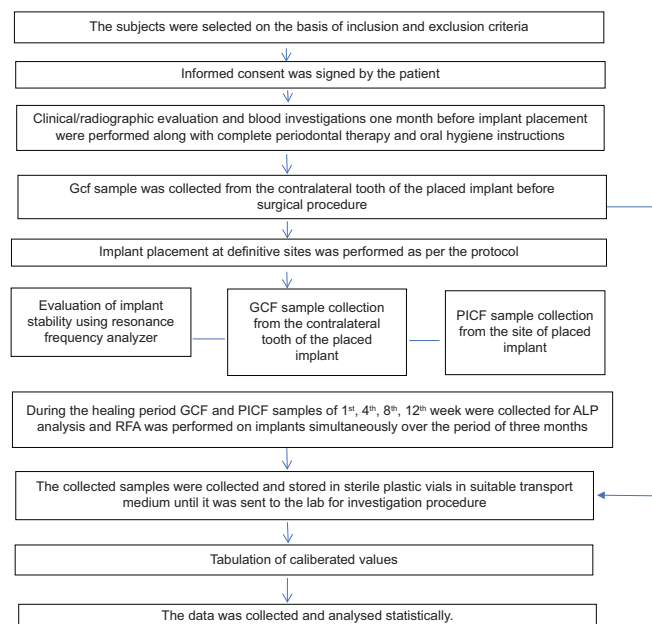
Biochemical analysis

After collecting gingival and PICF at the given time of healing weeks, testing for ALP was performed. The testing for ALP level was performed within 24 h because ALP

activity increases by 3–10% on standing at 25°C or 4°C for several hours. The enzyme is stable at 0–4°C for 2–3 days and “–25°C” for a month. For enzyme analysis, a kit manufactured by Transasia Biomedicals Ltd. was used to measure the activity of the enzyme. Colorimetric determination of ALP activity according to IFCC recommendation was done. Under optimized conditions, ALP presents in the sample catalyzes the following reaction:



Flow diagram



The data were obtained and tabulated into three groups: ALP level in GCF, ALP level in PICF, and RFA before and during the healing period of dental implants. Statistical analysis of data was done using Paired *t*-test and Pearson correlation coefficient test.

RESULTS

The highest mean was shown by ALP in the GCF sample of contralateral tooth during 12th week of implant placement followed by values of the 8th week, 4th week, on the day of implant placement, before implant placement, and 1st week, respectively.

The highest mean was shown by ALP during the 12th week after implant placement, followed by values in the 8th week, 4th week, on the day of implant placement, and 1st week (Table 1).

The mean values of ALP levels in PICF FOR 1st, 4th, 8th, and 12th-week post-implant insertion were highly significant with $P < 0.001$ (Table 2).

The highest mean value was shown by the RFA values of the 12th week, followed by values of the 8th week, 1st week, and

4th week and on the day of implant placement.

The mean values of RFA for 1st-, 8th-, and 12th-week post-implant insertion were highly significant with $P < 0.001$ (Table 3).

The mean values of the 8th week and 12th week were highly significant with $P < 0.001$ suggesting that ALP levels reach its peak when the mature bone formation process around the implant is progressing.

The mean values of 1st, 4th, 8th, and 12th week were highly significant with $P < 0.001$ which signifies that ALP can be used as a biological marker for monitoring implant stability at the time of implant placement and during the healing period. The ALP values dropped during the 1st week period suggestive of reduced osteoblastic activity. Thus, prosthetic loading should be avoided during this period.

Among these, the mean values of 1st, 8th, and 12th-week post-implant placement were highly significant with $P < 0.001$ suggesting that implant loading can be done at 1st week (immediate loading), 8th week (non-functional loading), and 12th week (conventional loading) and it must be avoided during 4th week of the healing period.^[9]

From the collected data, it can be inferred that these two entities are totally independent of each other and can be used as a valuable tool in measuring implant stability individually at the time of implant placement and during the healing period of implants.

The ALP levels in GCF and PICF on correlation with RFA were statistically non-significant on the day of implant placement, 1st, 4th, 8th, and 12th week post-implant placement with *r* values of –0.102, 0.177, 0.001, –0.046, 0.270, 0.045, 0.200, 0.031, –0.058, –0.129, respectively, and $P = 0.618$, 0.388, 0.998, 0.823, 0.182, 0.825, 0.328, 0.879, 0.778, and 0.529, respectively (Table 4).

This signifies that RFA and ALP levels in GCF and PICF are independent of each other and both can be used separately to measure implant stability and both are reliable tools to measure implant stability before, during, and after the implant placement.

DISCUSSION

For proper anchorage of implants inside the bone, the implant itself along with the bone plays a key role. The third important parameter is the metabolic activity occurring at the site during the formative stage. The stability of the external component is provided by the mechanical interlocking of the implant. Bone biomarkers are products of the bone remodeling process and include bone formation biomarkers, bone resorption biomarkers, and regulators of bone turnover. Recent advances in understanding bone metabolism and metabolic bone disease have expanded the use of biochemical bone turnover markers.

Biomarkers of osteogenesis include total ALP, bone-specific

Table 1: Mean values of ALP levels in GCF

Time	Mean	Standard error of mean	Standard deviation	Minimum	Maximum	P-value [#]
Before implant placement	4.8342	0.39997	2.03945	2.00	10.40	-
On the day of Implant placement	4.8342	0.39997	2.03945	2.00	10.40	1.000
1 st week	4.1485	0.25191	1.28447	2.80	7.70	0.143
4 th week	6.6346	0.49818	2.54023	3.20	14.00	0.009*
8 th week	8.0346	0.46197	2.35558	3.80	14.80	<0.001**
12 th week	8.8462	0.45276	2.30863	5.00	15.00	<0.001**

[#]Paired t-test; * $P < 0.05$; Significant; ** $P < 0.001$; Highly significant. ALP: Alkaline phosphatase, GCF: Gingival crevicular fluid

Table 2: Mean values of ALP levels in PICF

Time	Mean	Standard error of mean	Standard deviation	Minimum	Maximum	P-value [#]
On the day of Implant placement	7.7423	0.40093	2.04434	3.00	13.00	-
1 st week	4.6277	0.31693	1.61605	1.30	8.00	<0.001**
4 th week	6.9869	0.39851	2.03202	2.40	12.00	<0.001**
8 th week	9.4538	0.45581	2.32417	3.90	13.00	<0.001**
12 th week	10.5269	0.45264	2.30800	5.70	14.00	<0.001**

[#]Paired t-test; * $P < 0.05$; Significant; ** $P < 0.001$; Highly significant. ALP: Alkaline phosphatase, PICF: Peri-implant crevicular fluid

Table 3: Mean values of RFA

Time	Mean	Standard error of mean	Standard deviation	Minimum	Maximum	P-value [#]
On the day of implant placement	64.7692	1.94328	9.90882	43.00	83.00	-
1 st week	71.3846	1.30452	6.65178	50.00	84.00	<0.001**
4 th week	65.1923	1.36891	6.98008	48.00	77.00	0.774
8 th week	72.1923	1.16753	5.95328	55.00	83.00	<0.001**
12 th week	76.7308	1.09903	5.60398	65.00	88.00	<0.001**

[#]Paired t-test; * $P < 0.05$; Significant; ** $P < 0.001$; Highly significant, RFA: Resonance frequency analysis

Table 4: Correlation between ALP GCF, ALP PICF, and RFA Quotient during healing period

Time	Alkaline phosphatase level	RFA	
		r-value	P-value
At the time of implant placement	GCF	-0.102	0.618
	PICF	0.177	0.388
1 st week	GCF	0.001	0.998
	PICF	-0.046	0.823
4 th week	GCF	0.270	0.182
	PICF	0.045	0.825
8 th week	GCF	0.200	0.328
	PICF	0.031	0.879
12 th week	GCF	-0.058	0.778
	PICF	-0.129	0.529

Pearson correlation: * $P < 0.05$; Significant; ** $P < 0.001$; Highly significant. ALP: Alkaline phosphatase, GCF: Gingival crevicular fluid, PICF: Peri-implant crevicular fluid, RFA: Resonance frequency analysis

ALP, osteocalcin (OC), procollagen type 1 N-terminal propeptide, and procollagen type 1 C-terminal propeptide. Biomarkers of bone resorption are hydroxyproline, hydroxylysine, deoxypyridinoline, pyridinoline, bone sialoprotein, osteopontin, tartrate-resistant acid phosphatase 5b, carboxy-terminal cross-linked telopeptides of collagen type 1, amino-terminal cross-linked type 1 telopeptide

collagen, and cathepsin K.^[1] The regulators of bone turnover are RANKL, osteoprotegerin, dickkopf-1, and sclerostin.

A sensitive marker of osteogenesis, the use of OC measurements in clinical practice, is limited by assay variability, sample instability, and high biological variability. Hence, ALP is a reliable and more useful marker due to ease of histochemical analysis.

GCF is a serum derivate transudate in health or an exudate in disease which contains microorganisms, their products, and host derived substances (cytokines, enzymes, immunoglobulins, tissue degradation products, and immune system cells). Similarly, PICF, which is located in the peri-implant crevice, contains valuable biological material that can be used for diagnostic purposes. GCF from the contralateral tooth was used as a control group. Mean values for GCF during the healing period showed a significant increase from 4th week to 8th week and raised gradually reaching its peak at the 12th week.

Pandian *et al.* stated that ALP is thought to be involved in the first step of mineralization, lower pull-out values with high ALP activity could indicate primary mineralization with ongoing bone-tissue remodeling in the interface, whereas high pull-out values with low ALP activity most probably indicate a steady-state bone conditions with a more mature bone-implant interface.^[10]

In this study, mean values of ALP levels in PICF showed a significant drop in the values from the day of implant insertion to 1st week, and a gradual rise in the values was observed from 1st to 4th week followed by a significant gradual rise in the values of 8–12th week signifying the formation of bone around dental implants.

On the contrary, Bolger, in his study, stated that the relationship of this enzyme with the process of calcification and bone formation is not clear. ALP appears to be involved with the elaboration of the matrix rather than with the process of mineralization in osteogenesis.^[11]

Along with biomarkers, the stability or mechanical engagement of the implant with bone is an important parameter for osseointegration. The implant stability can be evaluated with various aids, but the RFA, being a non-invasive method is a reliable indicator to assess stability. Studies have shown that a higher RFA value indicates a more successful implant treatment and a lower risk of future failure. Conversely, low or declining RFA levels indicate a higher risk of implant complications, although the exact RFA threshold has not yet been identified.^[12] According to Brånemark *et al.*, decreased stability corresponds to stages of bone remodeling. This stage varies between weeks 2nd and 4th.^[13]

In this study, mean ISQ values ranged between 64.6 and 76.73. The initial mean ISQ values were 64.7692, these findings were harmonizing with the results of the previous study by Tallarico *et al.* that the macro-design of dental implant affects the primary anchorage and high ISQ value is a factor in making decision on immediate loading of dental implants.^[14] Bishop *et al.* and Talanico *et al.* found a mean post-operative ISQ of 60.3 and 62.1, respectively, and the mean post-operative ISQ in this study was 64.76.^[15]

The highest mean ISQ value was observed in the 12th week with a value of 76.7308 closely followed by the 8th and 1st week with values of 72.1923 and 71.3846, respectively. A short period of decreased ISQ values was observed after the 1st week till the 4th week with values of 71.3846 and 65.1923.

Lopez *et al.* concluded that while the theory behind RFA is correct, the technique cannot provide significant value that can determine implant success, failure, or long-term prognosis. Therefore, bone quality classification and insertion torque should be considered as parameters.^[16]

Correlation between mean ALP levels in GCF, PICF, and RFA showed that these two entities are totally independent of each other and both can be used to measure implant stability depending on which loading of implants can be assessed. According to a study by Al Nawas *et al.* 1996, ALP is an enzyme involved in bone metabolism, has been shown to be significantly elevated in active sites as compared to inactive sites.^[17]

According to Brunski *et al.* 2009 after the installation of implants, the primary mechanical stability decreases rapidly,

whereas secondary biological stability increases slowly. This phenomenon has been termed a “dip” (or drop or gap) instability.^[18,19] This dip can also be appreciated in levels of ALP during the 2nd and 3rd week; hence, loading of implants during this period is associated with a risk of unfavorable micromovement.

The purpose of the present “*in vivo*” study was to determine the level of ALP in GCF, after the analysis, it was observed that the ALP level decreases during the 1st week and increases in the 8th week, reaching the maximum peak during the 12th week suggesting that ALP decreases when acid phosphatase increases due to resorptive activity of the osteoclasts and its level increases as mature bone formation progresses over the healing of the implant site.

The levels of ALP in PICF after implant placement and during the healing period showed that the biomarker analysis is a reliable tool for measuring implant stability which, in turn, helps in determining the loading protocol of the implant and its successful prosthetic rehabilitation.

The stability of the dental implant at the time of insertion and during the healing period was also determined, using the non-invasive and mechanical method of RFA. The results showed a dip during the 4th week so it must be ensured that loading is avoided during this period. There was an increase in ISQ value during the 1st week and the values reach the maximum in the 12th week after seeing a gradual rise during the 8th week.

The comparative analysis between levels of ALP and ISQ value of RFA predicted that there is no statistical correlation between the two. Similar findings were observed by Tirachaimongkol *et al.* in his study, where he observed that ISQ values were not related to the level of biomarkers. Similar results were observed by Zhou *et al.* in his study.^[20]

Thus, it can be stated that the two entities are completely independent of each other as one is a biological parameter and the other one is a mechanical parameter. However, both methods are reliable tool's for assessing and measuring implant stability.

CONCLUSION

From the collected data, it can be inferred that these two entities are totally independent of each other and can be used as a valuable tool in measuring implant stability individually at the time of implant placement and during the healing period of implants.

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