Comparative analysis of serum titanium levels in patients with healthy dental implants and patients with peri-implantitis: A cross sectional prospective study

MAHANTESHA SHARANAPPA[1], VIBHA SHETTY[2], KRANTI KONUGANTI[1], SHOBHA SUBBAIAH[3], GREESHMA CHANDRASHEKHAR[1] and BABASHANKAR ALVA[2]

Departments of [1]Periodontology and [2]Prosthodontics and Implantology, Faculty of Dental Sciences, M S Ramaiyah University of Applied Sciences, Bangalore, Karnataka 560054; [3]Department of Periodontology, Oxford Dental College, Bangalore, Karnataka 560078, India

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Abstract. Titanium dental implants can last for more than two decades in the oral environment. The corrosion of the implant surface can release metallic particles or ions into surrounding tissue. The metallic constituents, such as titanium in human blood serum samples of patients with dental implants have not yet been extensively studied. The present study thus compared titanium serum levels before and after the placement of dental implants, and also compare the levels in patients with healthy implants and those with peri-implantitis. The present study comprised of patients in group 1 selected for dental implant surgery and patients in group 2 diagnosed with peri-implantitis. Each group comprised of 60 patients. Serum titanium levels were measured from blood samples obtained from patients in group 1 at three different intervals (at 1 month prior to implant surgery, and at the 4th and 8th month after successful loading) and from patients in group 2 during the course of peri-implantitis by inductively coupled plasma-mass spectroscopy. Statistical analysis was performed for the obtained data. The results revealed increased serum titanium level sat the 4th month post-implant placement (2.39 mg/dl) and in patients with peri-implantitis (2.94 mg/dl); both levels differed significantly (determined using ANOVA) from the pre-surgical estimation data of serum titanium levels (1.79 mg/dl). On the whole, understanding the association between titanium corrosion and peri-implantitis is vital for enhancing the long-term success and safety of dental implants. Additional research is required to investigate these links and develop potential strategies that can be used to protect the well-being of patients with dental implants.

Correspondence to: Dr Mahantesha Sharanappa, Department of Periodontology, Faculty of Dental Sciences, M S Ramaiyah University of Applied Sciences, New BEL Road, MSR Nagar, Bangalore, Karnataka 560054, India
E-mail: mahanperio@gmail.com

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Introduction

The use of titanium implants has increased substantially in dentistry to replace natural teeth due to their high biocompatibility. Titanium or titanium admixtures are the usual constituents of titanium implants, given their mechanical properties and biocompatibility. There is evidence to indicate that titanium dental implants can endure exposure to the oral environment for more than two decades (1). The biocompatibility exhibited by dental implants maybe attributed to the formation of a titanium dioxide layer that prevents direct contact between the implant and the biological environment. This, in turn, reduces the potential for metal reactivity. The success and durability of dental implants are contingent upon their integration with both hard and soft tissues (2). Currently, scholars are focusing on diverse approaches to expedite the process of osseointegration of dental implants and to augment the surface area of implant-bone contact. To achieve this, the osseointegration surface areas are enlarged by creating irregularities on the surfaces of materials used for dental implants (3). The examination of the interactions of dental implants with biological tissues involves the use of a titanium dioxide (TiO_2) layer. When subjected to loading conditions, the TiO_2 layer is susceptible to damage during movement between the implant and bone tissue, resulting in implant corrosion and consequential weakening. Furthermore, corrosion can trigger the release of minute metallic particles or ions into the surrounding living tissues (4). A previous study revealed that the micromotion of the abutment under cyclic loading may produce wear particles of varying sizes between 2 and 80 nm in conical dental implant systems. Wear debris comprises titanium particles that are recognized to elicit a macrophage response (5). Metal nanoparticles have been widely acknowledged for their ability to trigger an inflammatory response through their immunomodulatory potential. This potential is primarily exerted at the macrophage level and is characterized by an escalation in DNA damage, protein carbonylation, lipid

per-oxidation, oxidative stress, and a reduction in superoxide dismutase activity, total glutathione levels and total antioxidant capacity catalase (3).

Since the seminal study by Ferguson *et al* (6), the generation of metal debris from joint replacement surgeries has been a significant concern within the field of orthopedic surgery. The surface oxide film layer of a metallic object implanted within the human body may undergo disruption or degradation over time due to spontaneous mechanical or electrochemical corrosion. This particular interaction can yield chemically reactive metallic byproducts, which may prompt the discharge of metal into the systemic circulation (6,7). Bianco *et al* (8) investigated the dissimilarity between the levels of titanium in the serum and urine of rabbits and Gopi *et al* (9) investigated the serum level of titanium in humans before and following implant insertion. No noteworthy elevation was observed in either case. A comprehensive extensive analysis of the metallic constituents in human blood serum has yet to be performed, at least to the best of our knowledge. It is uncertain whether the discharge of titanium or titanium particles from dental implants may have an impact locally or systemically. The present study thus aimed to compare the titanium serum level before and after the placement of dental implants, and to compare the level of serum titanium in patients with healthy dental implants and in those with peri-implantitis.

Patients and methods

Study design. A null hypothesis was developed for the present prospective quasi-experimental study stating that there would be no alteration in the serum titanium level of patients before and after implant placement either in health or disease. The present study was carried out at the Department of Periodontics from 2019 to 2022 and was approved by the M S Ramaiyah University, Faculty of Dental Sciences Institutional Ethical Review Committee (EC-19/12-F-FDS). The study design consisted of two groups of 60 patients in each, including both males and females with an age range of 23-47 years. Group 1 comprised healthy individuals seeking implant placements and group 2 included patients with peri-implantitis who had implants placed >6 months prior. A well-informed written consent was obtained from all the patients informing them about the study and post study publication of data and any related images.

Sample size calculation. The statistical software G*Power (version 3.1) developed by Franz Faul at the University of Kiel (Kiel, Germany) was employed to determine the appropriate sample size for the present study, with a type 1α error rate of 0.05 and a power of 90% (9). Ultimately, a sample size of 53 was selected; however, in anticipation of potential sample attrition, the sample size was increased to 60. As per the sample size calculation each group of the study comprises of 60 patients contributing to total population of 120.

Inclusion criteria. The inclusion criteria used in the present study were the following: Group 1 (healthy individuals): i) Partially edentulous patients; ii) Periodontally healthy patients; iii) Patients with appropriate inter-occlusal distance for the placement of the implants; iv) Patients with no history of metal allergies. Group 2 (patients with peri-implantitis):

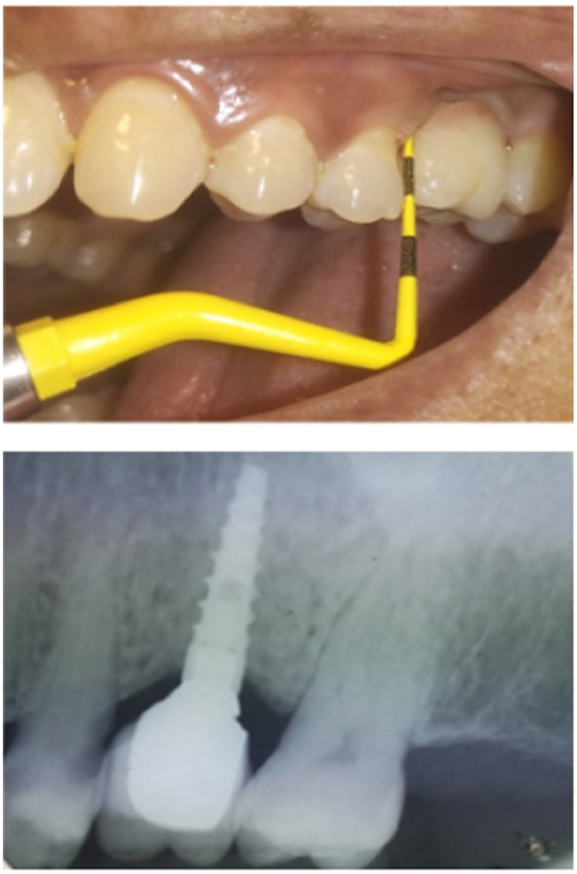

Figure 1. Clinical and radiological assessment of a patient with a healthy implant. (A) Periodontal pocket depth <2 mm. (B) Absence of bone loss.

i) The post implant period should be >6 months, but <2 years; ii) implants having probing depths ≥5 mm; iii) Bleeding on probing and/or suppuration; iv) Bone loss ≥2 mm was considered in the peri-implantitis group.

Exclusion criteria. The following exclusion criteria were used: i) Patients using systemic or local antibiotics over the last 3 months; ii) Immuno-compromised patients, who had received chemotherapy or radiotherapy; iii) Pregnant and lactating women; iv) Patients with a smoking habit.

Method of assessment of peri-implantitis. The determination of peri-implantitis diagnosis was established through the evaluation of clinical and radiologic criteria, in accordance with existing recommendations (10). Specifically, implants with probing depths ≥5 mm, accompanied by bleeding upon probing and/or suppuration, as well as a bone loss ≥2 mm, were diagnosed with peri-implantitis (Figs. 1 and 2).

In order to determine the diagnoses, it was necessary to obtain a consensus from three authors (MS, VS and KK) who worked independently. Furthermore, supplementary information regarding the age, sex, smoking habits and diabetes status of the study subjects was also documented and presented in Table I.

Methodology. Blood serum samples were collected from the patients of group 1 at three different intervals [at 1 month prior to implant placement, at the 4th month after the surgical phase

Table I. Descriptive characteristics of the study groups.

	Group 1	Group 2	P-value
Sample	60	60	
Male	32	35	0.581[a]
Female	28	25	
Age in years (mean ± SD)	37±8.6	39±9.2	0.221[b]
Smoking	0	0	NA
Diabetes	0	0	NA

Data were analyzed using a [a]Chi-squared test and [b]independent t-test.

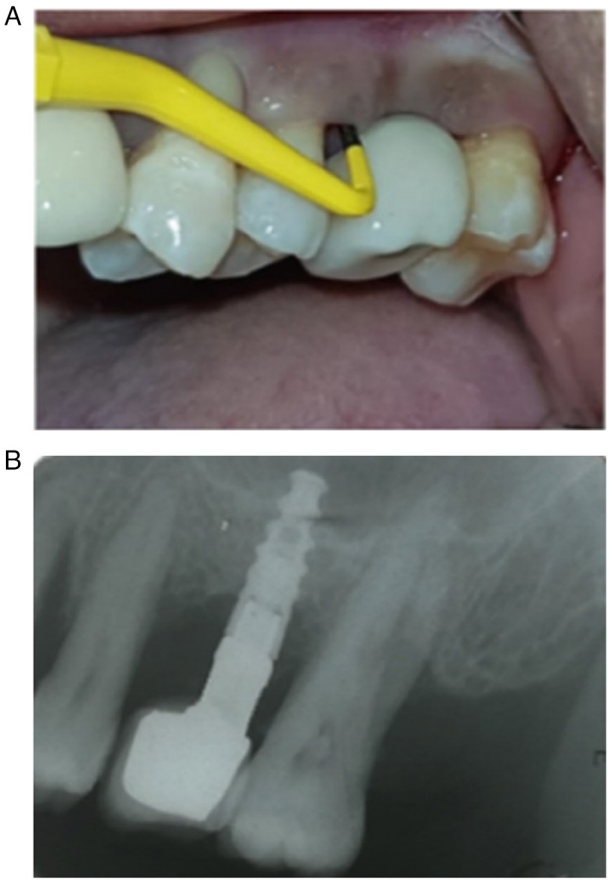

Figure 2. Clinical and radiological assessment of a patient with peri-implantitis. (A) Periodontal pocket depth >5 mm. (B) Radiographic bone loss >2 mm.

(loading of implant) and at the 8th month after the surgical phase (loading of implant)], and from the patients in group 2, at the time course of peri-implantitis.A total of 2 ml blood was withdrawn from the anterior cubital fossa of the patients. Blood samples were stored at -20˚C (Fig. 3). Following the collection of whole blood, it was left undisturbed in the vacutainer, allowing it to clot at room temperature. Centrifugation was performed at 271-542 x g for 20 min in a refrigerated (-4˚C) centrifuge. The resultant serum samples were obtained and analysis for titanium was performed using inductively coupled plasma-mass spectrometry (ICP-MS), Thermo Scientific ICAP 7000 series (Thermo Fisher Scientifc, Inc.) with a detection

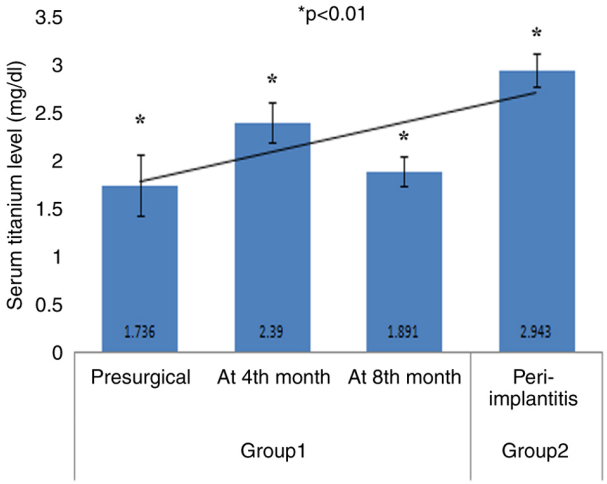

Figure 3. Graph illustrating the trend for increasing serum titanium levels in the study groups. *P<0.01.

limit of 0.5 ng at the M S Ramaiah Advanced Drug Testing Laboratory, Bangalore, India.

Statistical analysis. The normality of the obtained dataset was examined using the Shapiro Wilk test. After stating the descriptive analysis for all the groups, the Chi-squared test was applied for ordinal data and an independent t-test was applied for the comparison of mean age between the groups. The multiple group comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. A P-value <0.05 was considered to indicate a statistically significant difference. All statistical analyses were performed using SPSS software version 22 (IBM Corp.).

Results

The present study included 120 patients, comprised of 53 females and 67 males. The age range was 23-47 years with an average value of 40±8 years. In the present study, no statistically meaningful variance was observed as regards the demographic characteristics of the participants enlisted for the research, suggesting the absence of any bias in the selection process based on age and sex (Table I).

The analysis of the aggregated data revealed an increasing trend line in serum titanium levels in the patients in groups 1 and 2 (Fig. 3). Patients with peri-implantitis (group 2) exhibited higher mean serum values (2.94±0.17) compared to patients in group 1. In the patients in group 1, the highest mean serum titanium level (2.39±0.20) was observed at 4th month after the loading procedure (Fig. 3 and Table II). The comparative analysis of the groups using one-way ANOVA revealed a significant difference (P=0.001) and intra-group comparisons with Tukey's post hoc test revealed significant differences between all parameters examined, apart from difference in the serum titanium level between the pre-surgical time point and the 8th month, and between the4th month and 8th month (Tables III and IV).

Discussion

Since ~1981, titanium has been utilized for the construction of dental implants. The primary alloys employed are

Table II. Descriptive analysis of estimated serum titanium values in the study groups.

	Group 1 (n=60)			Group 2 (n=60)
Parameter	Pre-surgery	At the 4th month	At the 8th month	Peri-implantitis
Mean (mg/dl)	1.73	2.39	1.89	2.94
Standard deviation	0.31	0.20	0.14	0.17
Shapiro-Wilk test	0.94	0.91	0.92	0.94
P-value of Shapiro-Wilk test	0.005[a]	0.001[a]	0.001[a]	0.009[a]

[a]P<0.05, statistically significant difference.

Table III. Intergroup comparison of mean scores obtained using one-way ANOVA.

Cases	Sum of squares	df	Mean square	F value	P-value
Group 1 vs. group 2	43.808	3	14.603	377.705	<0.001[a]
Residuals	6.843	177	0.039		

[a]P<0.05, statistically significant difference.

Table IV. Intra-group comparisons determined using Tukey's post hoc test.

Group	Comparison made, vs.	Mean difference	SE	t value	P-value
Group 1 pre-surgical	Group 1 at the 4th month	-0.654	0.036	-18.218	<0.001[a]
	Group 1 at the 8th month	-0.162	0.036	-1.313	0.108
	Group 2 peri-implantitis	-1.207	0.036	-33.623	<0.001[a]
Group 1 at the 4th month	Group 1 at the 8th month	0.032	0.036	0.905	0.367
	Group 2 peri-implantitis	-0.553	0.036	-15.405	<0.001[a]
Group 1 at the 8th month	Group 2 peri-implantitis	-0.585	0.036	-16.31	<0.001[a]

[a]P<0.05, statistically significant difference.

commercially pure titanium (cpTi) and Ti-6Al-4V, both of which exhibit clinical success rates of up to 99% after a decade. These alloys possess biocompatibility when in contact with bone and gingival tissues, and have the ability to undergo osseointegration (9).

The corrosion behavior of metal implants is a crucial determinant of their biocompatibility. This is due to the potential detrimental effects resulting from the release of metal ions during the corrosion process. The tissue in the immediate vicinity of the implant, as well as the systemic environment, can be affected by these factors, potentially leading to allergic reactions. The presence of an oxide layer on the surface of the implant significantly influences the outcome of osseointegration. The utilization of dental implants may result in elevated levels of titanium in both the bloodstream and serum (11,12).

Gopi *et al* (9) conducted an assessment on the liberation of titanium, aluminium and vanadium from dental implants through a comparison of the serum concentrations of these ions prior to and following surgical procedures. Notably, a marginal variation was observed in the post-operative levels of titanium (2.31 mg/dl) in relation to the preoperative levels (2.28 mg/dl), without any statistically significant difference (P>0.5) (9). The present study assessed significant differences in the concentrations of titanium in the bloodstream before (1.79 mg/dl) and after (2.39 mg/dl) the 4thmonth of the loading of the implant, and the findings obtained were not in accordance with those of the study conducted by Gopi *et al* (9). Another study demonstrated a comparable lack of significance in the association between the average serum concentration of titanium at the beginning, after 8 weeks and after 6 months, with values of 2.39, 2.35 and 2.38 mg/dl, respectively (13). The present study also found no significant difference in the titanium level between the pre-surgical phase and at the 8th month of post-loading of the implant. This indicates that serum titanium levels significantly increase immediately after the loading of the implant; however, with time, the concentration decreases.

The release of titanium particles from the surface of the implant has a detrimental effect on both the nearby and far-reaching tissue, as it infiltrates the surrounding tissues

and enters the bloodstream (14). In the localized region of the dental implant known as the peridontium, the presence of titanium particles can trigger an inflammatory condition known as peri-implantitis, characterized by an escalation in inflammatory mediators, such as macrophages, cytokines, TNF-α and IL-6. Previous studies have assessed the concentration of titanium and inflammatory mediators in serum; however, no substantial findings have been attained (15,16). The present study, on the other hand, revealed a statistically significant difference in the serum concentration of titanium between individuals with uncompromised dental implants and those experiencing peri-implantitis.

Previous research has demonstrated the existence of titanium particles in the tissues surrounding dental implants. Nevertheless, no conclusive statistical evidence has been presented to establish a connection between dissolved titanium and peri-implantitis (17). The present study assessed the levels of titanium in the serum of patients with both healthy implants and implants affected by peri-implantitis. The findings obtained demonstrated significant differences, which is in accordance with the findings in the study by Olmedo *et al* (14).

Although the occurrence of inflammation is observed as a healing response promptly following the loading of an implant and is accompanied by heightened levels of titanium in serum (10), a similar response can also manifest in cases of peri-implantitis. During peri-implantitis, macrophages that are recruited engulf wearable titanium particles, resulting in an elevation of titanium levels in serum (14,16). The potential of titanium particles that have undergone corrosion to induce an immune response may result in inflammation of the periodontium and the subsequent degradation of bone tissue. During the process of immune activation, a variety of inflammatory cytokines is discharged, which encompass granulocyte-macrophage colony-stimulating factor, prostaglandin, TNF-α, IL-1β and IL-6. The catalyst responsible for this activation is the presence of titanium particles, which subsequently initiates the activation of the NLRP3 inflammasome, ultimately leading to the release of a mature IL-1β (18). The primary focus during the management of peri-implantitis was previously centered on mitigating inflammation. However, the emphasis should be on reducing the corrosion of titanium implants.

While the serum titanium level does not reach toxic levels in instances of dental implants, it is important to note that these titanium particles have the potential to be transported through the bloodstream to various regions of the body, thereby inducing toxic consequences (19). The exposure of titanium in dental implants to fluoride ions can occur through mouth rinses, toothpastes, drinking water or food. Consequently, the utilization of fluoride as a potential confounding factor should be taken into consideration in forthcoming confirmatory investigations that aim to evaluate the connection between titanium corrosion and peri-implantitis (20,21). In order to mitigate the leaching of titanium particles, a previous study was conducted using an aqueous solution of lactic acid and phosphate-buffered saline. However, it was discovered that there was no discernible connection between the augmentation of surface roughness and the release of ions, both in experimental and biological circumstances (20).

The present study has certain limitations, which should be mentioned. The present study utilized the ICP-MS technique to evaluate the serum titanium level which present in minute amounts. Although a significant association between healthy implant and implants with peri-implantitis was obtained, the status of inflammation in soft tissues around the implant was not assessed. Further studies are thus warranted to evaluate the titanium level and inflammatory components in gingival tissues and blood serum.

In conclusion, understanding the complex association between titanium corrosion and peri-implantitis is crucial for improving the long-term success and safety of dental implants. Further research is required to explore these connections and potential mitigation strategies to ensure the continued well-being of patients with dental implants.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MS made a substantial contribution to the conception and design of the study, as well as in the critical reviewing of the manuscript. VS was involved in patient selection and in the design of the study, in the analysis and interpretation of the data, as well as in the critical reviewing of the manuscript. KK was involved in the analysis and interpretation of the data, as well as in the critical reviewing of the manuscript. SS was involved in the laboratory investigation and data segregation, as well as in the critical reviewing of the manuscript. GC was involved in data analysis and in the critical reviewing of the manuscript. BA was involved in implant surgery and patient selection along with the critical reviewing of the manuscript for important intellectual content. All authors have read and approved the final manuscript. MS and VS confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Institutional Ethical Review Committee of the Faculty of Dental Sciences (EC-19/12-F-FDS) at Ramaiah University of Applied Sciences Bangalore. Written informed consent was obtained from the patients for their participation in the present study.

Patient consent for publication

Written informed consent was obtained from the patients for the publication of the present study and any related images.

Competing interests

The authors declare that they have no competing interests.

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