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Araştırma/ Research Article

EVALUATION OF INFLAMMATORY CYTOKINE AND PLASMA TITANIUM LEVELS IN DENTAL IMPLANT TREATED PATIENTS

DENTAL İMPLANTLARLA TEDAVİ EDİLEN HASTALARIN INFLAMATUAR SİTOKİN VE PLAZMA TİTANYUM SEVİYELERİNİN DEĞERLENDİRMESİ

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ÖZET

Amaç: Bu çalışmada kanda Titanyum (Ti) dental implantların korozyon ürünlerinin ve de fizyolojik korozyonu etkileyen inflamatuar sitokin sekresyonunun Ti implantlar üzerine etkisinin değerlendirilmesi amaçlanmıştır.

Metod: Kırk hasta iki gruba ayrıldı. Group 1 Microtextured Ti yüzeye sahip Zimmer Tapered Screw-Vent implantlar ile tedavi edildi. Group 2 Hidroksi Apatit yüzeye sahip Implant Direct ScrewPlant implant sistemi ile tedavi edildi. Bütün implantlar mandibular posterior bölgeye yerleştirildi. İmplantasyon öncesi ve cerrahiden 4 ay sonar periferal kan örnekleri alındı ve analiz edildi.

Bulgular: Çalışma gruplarının hepsinde istatiksel olarak anlamlı olmasa da artmıştır ($p>0.05$). Bütün gruplar implant uygulaması öncesi ve sonrası serum Ti seviyeleri karşılaştırıldığında serum IL-1 beta ($p<0.05$) düzeyi dışında Ti, IL-6, IL-10, ya da TNF-alfa ($p>0.05$) seviyelerinde artma saptanmamıştır.

Sonuç: Serum Ti seviyesi ile implant uygulanması arasında bir ilişki saptanmamıştır.

Anahtar Kelimeler: Stokin, korozyon, dental, implant, titanyum

ABSTRACT

Aim: This study aims to evaluate the corrosion properties of Titanium (Ti) dental implants in blood and also, measure the inflamatory cytocine secretion to affect of physiological corrosion on Ti implants.

Method: Forty patients were divided to two groups. Group 1 was treated with the Zimmer Tapered Screw-Vent implant system which has a Microtextured Ti surface. Group 2 was treated with the Implant Direct ScrewPlant implant system which has a Hydroxy Apatite surface. All implants were inserted in posterior mandibular region. Prior to implantation and 4 months after the surgery, peripheral blood samples were taken and analyzed.

Results: The Ti levels of every member in the study group was increased, but not to a statistically significant degree ($p>0.05$). A comparison of pre- and post-dental implant applications in all study groups showed no differences for levels of serum Ti, IL-6, IL-10, or TNF-alpha ($p>0.05$) except for IL-1 beta ($p<0.05$).

Conclusion: No association was found between serum Ti level and implant application.

Keywords: Cytokines, corrosion, dental, implant, titanium

INTRODUCTION

Dental implant application is a very simple dental procedure in patients with normal bone volume and density. Endosseous implants have been used extensively in rehabilitation of edentulous patients. Besides providing retention, dental implants assist in reducing the impact of tissue borne edentulous prosthesis especially for the special needs elderly patients such as dementia.[1] There is now overwhelming evidence those dental implants should become the first choice of treatment for the tooth lost in the near future.

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Commonly used materials for dental implants or orthopedic applications are titanium (Ti) and Ti alloys because of such excellent characteristics as their chemical inertia, mechanical resistance, low density, absence of toxicity, resistance to corrosion and biocompatibility.[2]

The oral cavity tolerates wide ranges of pH and temperature. The disintegration of metal may occur through the action of moisture, atmosphere, acid or alkaline solutions, and certain chemicals. Further it has been reported that water, oxygen, chlorides, sulphur corrode various metals present in dental alloys. Pure Ti metal is high reactive with oxygen. Fresh Ti exposed to the atmosphere oxidizes extremely rapidly, forming a TiO_2 layer. Although, Ti has great resistance to corrosion in saline and acidic environments, because of the stability of this TiO_2 layer, it is not inert to corrosive attacks.[3]

Studies have examined the presence of Ti in the serum of patients or animals that had used Ti or Ti alloy implants for joint arthroplasty or plate fixation of fractures.[4-6] In hip arthroplasties and dental implants, TiO_2 has been found adjacent to Ti implants, in regional lymph nodes and in some instances remote organs.[5,7] Ti toxicity has been reported in several in vivo and in vitro studies.[1,7,8] These studies showed that metallic ions can be released to local and remote tissues and in the human body[9] have been associated with side effects such as clinical implant failure, osteolysis[10], cutaneous allergic reactions, renal lesions, hypersensitivity, carcinogenesis[11], peri-implant tissue accumulation[12], and remote-site accumulation.[7,13]

Information on reliable Ti baseline physiological levels in blood and organ tissues is still pending. Thus far, corrosion of Ti or Ti alloys implants is controversial. In addition, inflammatory mediators on implant corrosion are not well studied, yet these effects are critical to implant biocompatibility and osseointegration. In the current study, the corrosion properties of Ti implants were studied in blood and IL-1beta, IL-6, IL-10, and TNF-alpha secretion was measured to affect of physiological corrosion on Ti implants.

MATERIAL and METHODS

Agreeing to participate in this study were 40 patients (24 male/16 female; 49.80±12.80 years, range 22-83 years). The patients had been referred to the Department of Oral and Maxillofacial Surgery of the Istanbul University Faculty of Dentistry, because of tooth loss in various regions of their mouths. Informed written consent to participate in this study was obtained from all patients. This study followed the Declaration of Helsinki medical protocol and ethics. Ethical permission was obtained from Istanbul University's Faculty of Medicine Ethical Committee (29.06.2009, no=2019). Patients were selected using the following inclusion criteria.

- >18 years of age;
- Partial or total edentulism;
- No history of radiotherapy in the head and neck region;
- No history of reconstructive pre-prosthetic surgery or previous implant surgery;
- No pathology in alveolar bone
- No periodontal diseases or infected teeth.

Patients were excluded from the study if any of the following criteria were present: a history of systemic diseases that would contraindicate surgical treatment, pregnancy, and use of more than 10 cigarettes per day. Patients smoking <10 cigarettes per day were requested to stop smoking before and after surgery.

Study Design

All 40 patients received dental implant therapy in their posterior mandibular region. The patients allocated into the group 1 (n=22) and group 2 (n=18). Group 1 included 22 patients were treated with the Zimmer Tapered Screw-Vent implant system (Zimmer Inc., Carlsbad, USA) which has a Microtextured Titanium (MTX™) surface. Group 2 included 18 patients were treated with the Spectra-System® screw (Screw-Vent) implants (Paragon Implant Co., Encino, USA) which have a Hydroxy Apatite (HA) surface.

Prior to implant application and 4 months after the surgery, 10 cc peripheral blood samples were taken from both groups to check for levels of Ti and serum cytokine. The samples were analyzed at Istanbul University Institute for Experimental Medicine. Serum samples were stored in aliquots at -80 °C until analyses were performed.

Detection of Serum Ti Level

Serum Ti level was detected by using an inductively coupled plasma/mass spectrometry (ICP-MS) device (ELAN DRC II, Perkin Elmer, SCIEX, Inc, Shelton, USA). In this study, ICP-MS was considered one of the most powerful tools for the determination of Ti for three reasons. First, ICP-MS has ability to sense low concentration levels in biological fluids. Second, only a small sample volume (i.e., blood) is needed. Third, these kinds of studies require large number of samples. Multielemental and multi-isotopic capabilities, low detection limits, and relatively simple spectra make ICP-MS an almost ideal technique to carry out many studies related to metals in biological samples.[14,15] Normal serum Ti levels are <150 µg/L. For the samples in this study, the test sensitivity was 0.2 µg/L.

Detection of cytokine levels

Serum levels of IL-1beta, IL-6, IL-10 and TNF-alpha were quantitatively determined by an enzyme-linked immunosorbent assay (ELISA) designed with multiplex technology (BioSource International, Camarillo, USA).

The assay procedure was performed according to the manufacturer's instructions. The ranges of measurability of the standard curve for each cytokine tested were: IL-1beta = 3.9 to 250 pg/ml; IL-6=7.8 to 500 pg/ml; IL-10=7.8 to 500 pg/ml; and TNF-alpha=15.6 to 1000 pg/ml.

Statistical Analysis

Statistical analysis of the data was performed using the NCSS 2007&PASS 2008 Statistical Software (NCSS, LLC, Kaysville, USA) package. Data was analyzed using descriptive and analytical methods. Student t-test was used to evaluate the mutual distribution of categorical properties, nonparametric Mann Whitney-U test was used to compare the quantitative characteristics of the two sub-groups which lack of homogeneous distributions, and paired Sample t-test was used to compare parameters which have homogeneous distributions. Percentage, mean, and standard deviation properties were used as a descriptive statistics. Tests of significance were reported at the 0.05 level.

RESULTS

All 40 patients had adequate bone and 127 dental implants were placed with primary stability (86 in Group 1 and 41 in Group 2) in posterior mandible. Group 1 was composed of 12 male and 10 female patients with average age of 52.23±11.45 (range 42-65) years old, and group 2 was composed of 12 male and 6 female patients with average age of 54.27±13.62 (range 45-83) years old. Bone healing was uneventful in all cases; no problems were seen. Implant survival rate was 100%. No difference was found between groups according to systemic differences and implant length/width ratios.

For whole study group, mean pre- and post-implantation Ti levels were 120.99±26.75 Pg/ml and 127.94±42.68 Pg/ml, respectively. Group 1 Ti level was 119.41±22.36 Pg/ml and 133.21±51.94 Pg/ml before and after the implantation. Group 2 Ti level was 122.93±31.88 Pg/ml and 121.50±27.69 Pg/ml.

In the whole study group, no statistically significant differences were found for serum Ti, IL-6, IL-10, or TNF-alpha levels pre- and post-dental implant applications except of IL-1beta (Table 1). Also, no differences were found for Ti, IL-1 Beta, IL-6, IL-10, or TNF-alpha levels in Group 1 and no differences were found for Ti, IL-6, IL-10, or TNF-alpha levels in Group 2 except of IL-1 Beta (Table 2). A positive correlation was found for Ti and IL-6 levels with age (Table 3). No correlation was found between age and number of implants and Ti levels, but positive correlation was found among the number of implants and TNF-alpha levels (Table 4).

Table 1.Before and after implant application cytokine and Ti values

	Before implantation mean±SD	**After implantation mean±SD**	*Paired sampl t-test* **+p**
Titanium (Pg/ml)	120.99±26.75	127.94±42.68	***0.262***
IL-1 Beta (Pg/ml)	2.68±1.10	3.06±1.42	***0.033****
IL-6 (Pg/ml)	5.99±4.62	5.79±3.50	***0.746***
IL-10 (Pg/ml)	2.49±0.75	2.76±0.82	***0.109***
TNF Alpha (Pg/ml)	19.67±4.00	19.58±2.74	***0.846***

** p<0.05*

Table 2.Before and after implant application cytokine and Ti values according to implant system

	Paired sample t-test	**Zimmer (n:22) mean±SD**	**Direct n (n:18) mean±SD**	***+p** Student t-test*
Titanium (Pg/ml)	**Before**	119.41±22.36	122.93±31.88	***0.684***
	After	133.21±51.94	121.50±27.69	***0.395***
	p	***0.178***	***0.810***	
IL-1 Beta (Pg/ml)	**Before**	2.79±1.11	2.55±1.11	***0.509***
	After	2.85±1.08	3.32±1.76	***0.312***
	p	***0.768***	***0.008*****	
IL-6 (Pg/ml)	**Before**	5.78±4.70	6.24±2.94	***0.757***
	After	5.53±3.92	6.10±3.00	***0.618***
	p	***0.756***	***0.887***	
IL-10 (Pg/ml)	**Before**	2.39±0.60	2.62±0.91	***0.354***
	After	2.58±0.31	2.98±1.15	***0.134***
	p	***0.179***	***0.285***	
TNF Alpha (Pg/ml)	**Before**	19.50±4.65	19.88±3.15	***0.769***
	After	19.33±3.40	19.89±1.67	***0.531***
	p	***0.742***	***0.991***	

*** p<0.01*

Table 3.Association between age and Ti level before and after implant application

		Age	
		R	**P**
Titanium (Pg/ml)	**Before**	0.329	***0.038****
	After	0.340	***0.032****
IL-1 Beta (Pg/ml)	**Before**	0.091	***0.575***
	After	0.007	***0.966***
IL-6 (Pg/ml)	**Before**	0.445	***0.004*****
	After	0.436	***0.005*****
IL-10 (Pg/ml)	**Before**	-0.126	***0.439***
	After	-0.108	***0.509***
TNF Alpha (Pg/ml)	**Before**	0.279	***0.081***
	After	0.274	***0.087***

Pearson correlation analysis ** p<0.05* *** p<0.01*

Table 4.Evaluation of the number of applied implants and Ti and cytokine level changes

		The number of applied implants	
		R	**P**
Titanium(Pg/ml)	Before	0.201	***0.213***
	After	0.306	***0.055***
IL-1 Beta(Pg/ml)	After	-0.128	***0.431***
IL-6(Pg/ml)	After	-0.098	***0.548***
IL-10(Pg/ml)	After	0.064	***0.693***
TNF Alpha(Pg/ml)	After	-0.345	***0.029****

Spearman's rho correlation analysis ** p<0.05*

DISCUSSION

Corrosion, the process of interaction between a solid material and its chemical environment, leads to a loss of substance from the material, a change in its structural characteristics, or loss of structural integrity. During corrosion, casting alloys release elements into the body.[2]

The TiO_2 layer determines the chemical properties, the corrosion resistance and the interface chemistry of the metal. This protective layer is thought to decrease the carcinogenicity and toxicity of Ti.[2,16] Despite this process, evidence exists that Ti implants can result in both local and systemic disbursement of Ti.[2]

The corrosion of a material such as gold alloy elicits a bio-reaction similar to the bio-reaction to bacterial toxins. Clinical symptoms such as pain, swelling, inflammation, lyses, and necroses are common responses to these local toxic reactions. Obviously, if these reactions are not eliminated, the process, which would ultimately end with implant failure, is accelerated. How the corrosion process can be slowed or halted once it has started is unclear.[17]

Olmedo et al.[18] observed that TiO_2 particles were transported in blood by phagocytic monocytes and deposited primarily in the lungs, but also in the liver and spleen six months after intra-peritoneal injection. The current study found that serum Ti level increased in whole study group after implant application, but not to a statistically significant degree ($p>0.05$). Possible explanation of the Ti level increment was not enhance significant level was the implants had no prosthetic application in this study, because of the prosthetic design and occlusal force distribution could not standardized, and therefore, the implants did not have lateral and vertical chewing forces that may increases corrosion level. Also, Ti accumulated not only in blood, but also in other tissues such as the spleen and lungs.

Most of the studies have tried to quantify the Ti present in the blood or organ tissues of implanted patients. They used a wide range of atomic analytical techniques (ETAAS, ICP-OES, ICP-QMS, etc.), but most lack the sensitivity and selectivity required for such studies.[14,15] For example, Jacobs et al.[19] recently reviewed their experience on Ti serum determination in more than 750 individuals using ETAAS and

concluded that Ti basal levels should be below their Ti detection limit (DL; 2.1 ppb). Their values were lower than those reported by other authors using the same technique [23.9 ppb[20], 3 ppb[4]]. This sensitivity problem could not be overcome by using quadrupole-based ICP-MS, because of the serious isobaric and polyatomic interferences with every Ti isotope—especially important when analyzing biological samples—making matters even worse than when using ETAAS. Moreover, interference attenuation using collision/reaction cell technology has proved that ICP-QMS is insufficient to quantify Ti at the basal levels in complex samples.[2,21] We choose to an ICP-MS, the analytical technique of choice for the determination of Ti in biological samples, because of the possibility of measuring at medium resolution with low level for Ti.[2]

Ti generally is regarded as safe for an organism[22], but has been reported as having a biochemical action of increasing prostaglandin E2 or IL-1.[23] Brayda-Bruno et al.[24] showed that Ti had accumulated in the brain, lungs, liver, spleen, kidneys, and lymph nodes, although the amounts were negligible small and would not have caused any clinical problems.

IL-1beta, IL-6, IL-10 and TNF-alpha are associated with peri-prosthetic osteolysis and are major contributors to this phenomenon.[25] Messer et al.[26] demonstrated that the corrosion rates of implants were increased with higher inflammation rates. In this study we evaluated IL-1 beta, IL-6, IL-10 and TNF-alpha levels for detection of inflammation degree and we found that 4 months after the implantation, only the IL-1 beta level had increased. IL-1beta, a potent stimulator of bone resorption, has been implicated in the pathogenesis of periodontal destruction. In a study with refractory periodontitis patients, Lee et al.[27] found similar significant higher gingival crevicular fluid levels of IL-1beta, IL-6, and TNF-alpha in refractive periodontitis sites than in healthy sites. Uematsu and Deguchi[28] also detected a significantly higher level of IL-1beta, IL-6 and TNF-alpha in orthodontic tooth movement sites compared with healthy periodontal sites. Atilla and Kutukculer[29] reported significant levels of IL-1 beta and IL-6 from deep pockets compared to shallow pockets. In this study it was found that IL-1 beta increment was significantly higher in group 2 and hypothesize that in our study increased IL-1 beta results were associated with normal immunological response. The current study supported other studies on implants with HA surface, said that degree of breaking and dissolution is higher in implants with HA surfaces. In addition, we found that IL-6 and Ti values increased with age. Future determinations of metal concentrations in serum should thus span a longer period.

Frisken et al.[7] evaluated the levels of Ti in the spleen, liver, and lungs before and after single implant placement in sheep mandibles. They found no statistically significant differences. Previous studies[20,21,26] using self-tapping implants, however, have reported much higher experimental levels in the order of 99,000 to 432,000ng/gm in experimental sites using similar threaded screw implants but with Ti screw-taps compared to 40-300ng/gm. They explained these differences as deriving from a single implant in each animal as opposed to implantation of several units. In this way, in their study the total volume of Ti implant in contact with the tissues was minimized. In the current study, on the contrary, it was found that plasma Ti level was not associated with the number of applied implants.

CONCLUSION

This study suggest that Ti level was increased in whole study groups; however, statistically not significant. Further investigations are essential to clarify the toxicologic importance of Ti alloy dental instrumentation.

Conflict of interest statement

All contributing authors declare no conflict of interest.

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