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Research Article

PILOT STUDY OF SERUM PROINFLAMMATORY CYTOKINES LEVELS IN PATIENTS TREATED WITH DENTAL IMPLANTS

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A B S T R A C T

The study was designed to determine the sensitization to titanium allergens in patients treated with dental implants and in individuals with suspected or proven sensitization to dental materials, and the correlation between serum levels of interleukin 1, tumor necrosis factor alpha, interleukin-17 and interleukin-22 and implant loss. Skin patch testing with Ti, TiO_2, Ti nitride, and Ti oxalate decahydrate was performed. The measurement of serum cytokine levels was performed by ELISA kits. Quantification of allergen-specific IgE in patient serum was performed. The following statistics were used: Chi^2 test, Fisher Exact Test, Mann-Whitney test. No significant differences concerning the incidence of sensitization to titanium allergens between the studied groups were revealed. The incidence of sensitization to all allergens was low, highest to Titanium nitride, especially among patients with periimplantitis. No significant between the groups concerning the cytokine serum levels were established. Using Mann-Whitney U test, significantly higher values of TNF-α levels were established among the patients without implants and sensitized to dental materials. We suppose that elevated TNF-α levels reflect unspecific reaction of allergic inflammation rather than a diagnostic tool for implant loss. Summarizing the results, we can assume that patients with atopic allergy are more inclined for sensitization to titanium.

INTRODUCTION

Titanium (Ti) is a non-essential metal element with atom number 22, silver in color (Brown, 1997). Around 95% of the worldwide used Ti is not in his metallic form, but as TiO_2 - pigment, highly valued for his chemical stability, brightness, resistance to UV light and supposed low-toxicity (Maia, 2003). The properties of titanium also made him key material for the development of medical and dental implants with huge percent of success, mainly because of its high resistance to corrosion, low toxicity, very low allergic potential, and favorable biological response in contact with human tissue (Niinomi, 2008; Steinemann, 1998). It is believed that the favorable bio-response is due to the restricted ion liberation, stability of the formed alloys and restricted bio effects of the ions. In contact of the titanium with air oxygen, immediately a layer of titanium dioxide is formed with 4 mm thickness, which is powerful barrier against the metal decay.

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According to some authors, this chemical inert layer of titanium dioxide is responsible for the bio-features of the titan in the human body (Kasemo, 1983; Kaus *et al*., 1996).

Titanium and its alloys are successfully used and are standard materials development dental implants, made of trade titanium or titanium alloys. The layer of titanium oxide allows apposition of physiological liquids, proteins, hard and soft tissues to the metal surface (ADA Council on Scientific Affairs, 2003). Commercially pure titanium is used mainly for dental implants. Pure titanium consists of 99,5% titanium, 0,5% intermediate elements (carbon, oxygen, nitrogen, hydrogen, iron) and the ratio between these elements directly corresponds to the quality of the metal. The alloy Ti-6A1-4V was initially used in space industry, and now together with the commercially pure titanium (CpTi) are the most used materials for medical and dental implants (Keegan *et al*., 2007).

The existence and the risk of developing an allergy to Ti and TiO_2 are widely discussed. The reason is the growing number of reports in the scientific literature for adverse reactions to Ti-based alloys and extremely low frequency of positive reactions in patch-testing to Ti salts, especially TiO_2. The significant increase in the use of TiO_2 in personal care products and Ti-

based medical and dental implants makes it necessary for the safety assessment of Ti in terms of sensitization potential.

Titanium dental implants are usually well tolerated. A small proportion of patients suffer from periimplantitis and subsequent loss of the implant (el Askary *et al*., 1999). When clinical symptoms are obvious, preventive measures are often ineffective. Early intervention or the choice of alternative implant materials with thorough individual risk assessment can improve treatment outcome. Predicting the risk of treatment failure with titanium implants, the importance of individual characteristics is postulated repeatedly, particularly in relation to genetic features underlying in the individual response to inflammation (Alvim-Pereira *et al*., 2008).

In this study we aimed to determine the serum levels of the following pro-inflammatory cytokines mediating inflammatory and osteolytic processes in perimplants - interleukin 1 (IL-1) and tumor necrosis factor alpha (TNF-α) as well as interleukin-17 (IL-17) and interleukin-22 (IL-22) and manifestation of sensitization to titanium allergens in patients treated with dental implants, as well as in individuals with suspected or proven sensitization to dental materials.

MATERIALS AND METHODS

Study subjects

A total of 100 participants were included in the study, divided into four groups:

Main group - 49 patients treated with dental implants, of which:

- 26 without reactions of implant rejection and periimplantitis, and
- 23 - with diagnosed perimplantitis.

Control group - 51 individuals, including:

- 32 patients with suspected or diagnosed sensitization to dental materials and history for allergies, and
- 19 healthy individuals without history for allergy, of different gender, age and occupations.

The study was approved by the Medical Ethics Board at Medical University of Sofia. All the participants were informed about the purpose of the study and gave their written informed consent.

Diagnostic assays

Skin patch testing with Ti (10% pet.), TiO_2 (10% pet.), Ti (III) nitride (5% pet.), and Ti (III) oxalate decahydrate (5% pet.) - Chemotechnique Diagnostics was performed according to the Jadassohn & Bloch classical methods for diagnosis of contact allergy, by placing the allergens in IQ-Ultra hypoallergenic patches of Chemotechnique Diagnostics (IQ Chambers®,Vellinge, Sweden). Lack of anti-allergic medication constituted a mandatory condition before placing the patches and during the testing. Patches with allergens were applied on the back of the tested individuals; reading of the test was performed on day 2, several hours after removing the patches, with control revision on day 3. Interpretation of reaction sites was based on the method and the interpretation key recommended by the International Contact Dermatitis Research Group (ICDRG).

The measurement of serum levels of IL-1α, TNF-α, IL-17 and IL-22 was performed by eBioscience (USA) competitive ELISA kits (Prod. No.: BMS243/2CE; BMS223/4CE; BMS2082; BMS2047), according to the manufacturer's instructions.

Quantification of allergen-specific IgE to a panel of 10 inhaled and 10 nutritional allergens in patient serum (Euroline Atopy) was performed by Euroimmun AG (Germany), according to the manufacturer's instructions, and the results were reported using EUROLineScan.

Statistical analysis. The statistics were calculated with SPSS 19.0. The following statistics available for cross-tabulation were used: Chi^2 test, Fisher Exact Test for statistical significance, Mann-Whitney non-parametric test. Values of $p<0.05$ were accepted as statistically significant.

RESULTS

The distribution of the investigated groups according to gender and age characteristics is presented below (Table 1).

Table 1 General characteristics of the studied groups

Group according to the presence of titanium dental implants and periimplantitis	Mean age (in years) ± SD	Gender		Total
		Women n	Men n	
Patients treated with dental implants without periimplantitis	50,38 ± 12,66	23	3	26
Patients treated with dental implants with periimplantitis	49,14 ± 7,99	17	6	23
Patients no treated with dental implants sensitized to dental materials	40,10 ± 15,39	22	10	32
Patients no treated with dental implants, no-sensitized to dental materials	22,33 ± 21,64	13	6	19
TOTAL	41,36 ± 17,55	75	25	100

The gender distribution was uniform. A significantly lower mean age was found in the group of patients without treatment with dental implants and sensitization to dental materials ($p = 0.029$).

Summarized data on the incidence of positive skin patch test reactions to the tested titanium allergens are presented in Table 2.

Table 2 Incidence of positive skin patch reactions among the studied groups

Allergen	Studied groups			
	Patients treated with dental implants without periimplantitis n (%)	Patients treated with dental implants with periimplantitis n (%)	Patients not treated with dental implants sensitized to dental materials n (%)	Patients not treated with dental implants no-sensitized to dental materials n (%)
Titanium	3 (11,5%)	3 (13,0%)	3 (9,4%)	3 (15,8%)
Titanium dioxide	3 (11,5%)	0 (0.0%)	3 (9,4%)	3 (15,8%)
Titanium(III)nitride	3 (11,5%)	6 (26,1%)	0 (0.0%)	3 (15,8%)
Titanium (III) oxalate decahydrate	0 (0.0%)	3 (8,8%)	3 (9,4%)	0 (0.0%)

No significant differences concerning the incidence of sensitization to the tested titanium allergens between the studied groups were revealed. However, despite the low incidence of sensitization to all the tested allergens in the defined by us groups, it was found to be highest to Titanium (III) nitride, especially in the group of patients treated with titanium dental implants and periimplantitis.

Below (Table 3), we present summarized data on the incidence of cases with serum cytokine levels above the cut-off values - 1.6 pg/ml for IL-1α, and 31.3 pg/ml for IL-22 in the studied groups. For TNF-α, the incidence of cases with especially high serum levels, over 119,1 pg/ml is presented. No data on IL-17

serum levels are presented, since no cases with serum levels above the cut-off values (31,3 pg/ml) were measured. No statistical significant between the groups defined by us were established.

Table 3 Incidence of cases with serum cytokine levels above the cut-off values

Cytokin	Studied groups			
	Patients with implants without periimplantitis n (%)	Patients with implants with periimplantitis n (%)	Patients without implants sensitized to dental materials n (%)	Patients without implants no-sensitized to dental materials n (%)
IL-1α	0 (0.0%)	3 (13,0%)	9 (28,1%)	0 (0.0%)
IL-22	0 (0.0%)	6 (26.1%)	9 (28,1%)	0 (0.0%)
TNF-α	0 (0.0%)	6 (26,1%)	6 (18.8%)	3 (15,8%)
TOTAL	0	15	24	3

Below (Table 4) are presented summarized data on mean ± SD, minimum and maximum TNF-α serum levels (pg/ml) in the defined groups. No significant differences between the defined groups were revealed during the statistical analysis.

Table 4 Mean (M±SD), minimum and maximum TNF-α serum levels (pg/ml) in the defined groups

Studied groups	TNF-α serum levels (pg/ml)		
	M±SD	Min	Max
Patients treated with dental implants without periimplantitis	34,68±14,42	16,8	59,6
Patients treated with dental implants with periimplantitis	119,59±174,8	21,8	509,9
Patients non treated with dental implants sensitized to dental materials	88,51±69,53	26,7	219,8
Patients non treated with dental implants no-sensitized to dental materials	55,27±49,72	23,0	154,3
TOTAL	75,20±95,18	16,8	509,9

Using the nonparametric statistics - Mann-Whitney U test, we established a significant difference regarding the mean TNF-α serum levels in the group of patients treated with dental implants without periimplantitis and the group of patients non treated with dental implants and sensitized to dental materials, the mean values being significantly higher in the latter group ($z = -2,399$, $p = 0,016$).

Euroline (Euroimmune) quantification of allergen-specific IgE to a panel of 10 inhaled and 10 nutritional allergens in patient serum (Euroline Atopy) was performed among a total of 23 patients with titanium implants, after estimation of their overall dental and allergological status. In 83% of them no sensitization to the allergens included in the panel was established. In 4 patients (17%), a significant increase in allergen-specific IgE (above 0.7 kUA / l) to one or more of the allergens included in the study panel was found. Data indicate that all the patients are sensitized to pollen allergens (grass, tree and weed). Elevated serum levels of some of the tested cytokines (TNF alfa and IL-22) were established in several of the patients above. Of interest is the fact that among 3 of the total of 4 patients with diagnosed IgE-mediated allergy, treated with titanium implants and with suspected or established sensitization to dental materials, and elevated serum levels of one or two of the examined cytokines manifested positive patch tests to different titanium allergens.

DISCUSSION

The diagnosis of allergy to Ti is usually based on patient history, clinical findings and the results of patch testing. However, low epidermal penetration of commercially available Ti salts makes patch testing insufficiently reliable (Forte *et al*., 2008). The results obtained in the present pilot study with the observed very low incidence of sensitization to titanium allergens confirm the statement above. Nevertheless, we can assume that titanium compound with highest sensitizing potential is Titanium (III) nitride.

Titanium particles with a diameter of 1-10 microns penetrate from implants in the connective tissue, which are potent stimulators of macrophages stronger than polyethylene, CoCr, ZrO2 and aluminum particles (Kaufman *et al*., 2008). Titanium particles were found in tissue macrophages and osteoclasts. It is understood that macrophages release IL-1 and TNF-α in phagocytosis of titanium particles mediating powerful inflammatory response. Together with their proinflammatory, IL-1 and TNF-α also have osteolytic effect. Furthermore, they contribute to the degradation of components of the extracellular matrix by metalloproteinases (Birkedal-Hansen, 1993). In short term, inflammation with moderate release of IL-1 and TNF-α has been shown to favor the primary bone regeneration, a process similar to the osseous integration of dental implants. Low level of inflammation expression is a factor favorable to the exit of implantation because osseous integration of implants depends on the adequacy of tissue repair and adequate immune response (Kronstrom *et al*., 2001). The strong or long-term release of IL-1 and TNF-α boosts both the inflammatory and the osteolytic processes that lead to an increased risk for developing severe periimplantitis and failure of implantation. Investigations indicate that both early- and late loss of an implant is associated with a significantly increased production of TNF-α and IL-1β as compared to controls. This data suggest that the individual inflammatory response to the titanium particles contribute to increased risk for both early- and late loss of an implant. This is the first study integrating genetic and functional analysis of IL-1β and TNFα production as diagnostic agents for treatment failure with titanium implants (Jacobi-Gresser *et al*., (2013).

Predicting the risk of treatment failure with titanium implants, the importance of individual characteristics is postulated repeatedly, particularly in relation to genetic features underlying in the individual response to inflammation (Alvim-Pereira *et al*., 2008). Most researches on the genetic basis of the failure of implantation showed significant correlations only when genetic and non-genetic risk factors are combined. Synergy between IL-1 polymorphisms and smoking on the incidence of implant loss was postulated (Jansson *et al*., 2005; Andreiotelli *et al*., 2008).

According to the results obtained in the present study, though the mean TNF-α serum levels were highest in the group of patients treated with dental implants with periimplantitis, using the nonparametric statistics we established that the mean values of this cytokine were significantly higher in group of patients non treated with dental implants and sensitized to dental materials if compared with the group of patients treated with dental implants without periimplantitis. We could suppose that elevated TNF-α serum levels reflect an unspecific reaction of allergic inflammation rather than a diagnostic tool related with implant loss.

Interleukin-17 (IL-17) and interleukin-22 (IL-22) are produced from a subset of newly defined T-cell line known as Th-17. IL-

17 relates to many inflammatory diseases, including. rheumatoid arthritis and asthma. The number of Th-17 cells and the expression of IL-17 were significantly higher in biopsies in positive patch test, regardless the nature of the antigen (Larsen *et al*., 2009; Oboki *et al*., 2008). IL-22 is an important mediator in the mucosal protection and has complex pro-inflammatory and anti-inflammatory and auto-immune effects. Patients with contact dermatitis to nickel have a significantly higher level of IL-22 in blood compared to the controls, indicating a possible involvement of IL-22 in the pathogenesis of human allergic contact dermatitis (Ricciardi *et al*., 2009).

The results obtained by us didn't confirm relation between IL-17 and IL-22 serum levels and implant loss. Elevated serum levels of some of the tested cytokines (TNF alfa and IL-22) were established in several patients treated with dental implants and possitive for allergen-specific IgE antibodies.

Euroline (Euroimmune) quantification of allergen-specific IgE give very good information of the allergic status of the patients studied. Additionally, the test can guide on type of atopic disease and the potential cross-reactivity between the pollen and the food allergens involved. Our data indicate that all the patients are sensitized to pollen allergens (grass, tree and weed) and once again confirm that pollen allergy is the most common among atopic individuals in Bulgaria and Europe.

Of interest is the fact that 3 of the total of 4 patients with diagnosed IgE-mediated allergy, treated with titanium implants, with suspected or established sensitization to dental materials, and elevated serum levels of one or two of the examined cytokines manifested positive patch tests to different titanium allergens.

CONCLUSION

Summarizing the results presented, we can assume that patients with atopic allergy are more inclined to develop sensitization to various dental materials and titanium. No significant differences in serum cytokine levels among patients with titanium implants were found to allow more definitive conclusions about their clinical importance to be done.

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