Research Article

Role of Advanced Glycation End Products (Ages) and Oxidative Stress in the Failure of Dental Implants

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Abstract

Introduction: In the last decade dental implant has become crucial beneficial modalities. Many clinical dental implant systems have been established which can be used as the individual form of therapy or it may function together with other dental treatment methods. In this study we highlighted the role of AGEs and oxidative stress in terms of lipid peroxidation (TBARS) in the progression of periimplantitis and in the failure of dental implants.

Material and methods: In this study we selected three groups for the investigation of TBARS and AGEs. The data consist of 10 subjects (7 M/3 F) aged between 40-60 years (average 50.0 ± 4.6). Teeth were extracted and then put in PBS solution before dry freezing at -80°C. Also collect the saliva of patients and also from healthy individual for the study of TBARS.

Results: The statistical analysis for oxidative stress and advanced glycation end product were performed using software SPSS (Version 17.0). The statistical difference in terms of lipid peroxidation (TBARS) and AGEs shows a significant difference between groups. This shows that increase in TBARS in saliva could lead to higher oxidative stress levels in periimplantitis and periodontitis groups than in healthy group. This difference can be explained on the basis of one-way ANOVA with post-hoc Bonferroni correction due to the small sample size. The level of significance was set at $p < 0.05$ for all tests. Results are expressed as mean absorbance value.

Conclusion: According to our study, periimplantitis as a multifactorial disease, in which glycation and oxidative stress play a role in terms of etiology and severity. This hypothesis could lead to new therapeutic strategies in periimplantitis, using antioxidant approach in addition to conventional treatments.

Introduction

In the last decade dental implant have become crucial beneficial modalities. Due to the high survival rate of osseointegrated root form dental implants has led to their acceptance as an alternative treatment. In spite of these achievements, however, over a 5 year period, 0 to 14.4% of the dental implants demonstrated peri-implant inflammatory reactions which were associated with crestal bone loss that may eventually lead to the loss of an implant [1]. There are three conceivable responds that may arise in host tissues after the installation of endosseous implants: (1) acute or chronic inflammatory progression that cause early implant failure; (2) the formation of connective tissue nearby implant, leading to osseointegration failure, and (3) living and functional bone tissue development around the implants, resulting in osseointegration [2].

Many clinical dental implant systems have been established which can be used as the individual form of therapy or it may function together with other dental treatment methods. Dental implants show similar combined success rates at 10 years after implantation if good quality techniques are used. The 10-year cumulative success degrees are about 88% for maxillary and 93% for mandibular implants [3], but some implants are lost as a result of primary failure or by loosening, a mode of failure resulting from implant movement or migration in the bone. Many important risk factors subsidize to this slackening, such as non-ideal implantation techniques, oral bacterial, bio-mechanical load, and use of non- completely biocompatible implants.

Advanced glycation end products (AGEs), is the products of non-enzymatic glycation and oxidation of proteins and lipids that store in various biological settings [4]. If the Advanced glycation end products (AGEs) is stored in the body they cause many harmful effects. AGEs can alter proteins and they directly damage the structure and metabolism of extracellular matrix. They act via their specific receptors called RAGE (receptor for advanced glycation end-products).

Maillard reaction is called the driving force of AGE formation because in 1912 Maillard first observed a browning reaction by heating glycine and glucose [5]. Advanced Glycation Endproducts are considered as a heterogeneous group of compounds that arise non-enzymatically by the reaction of reducing sugars and other α-carbonylic compounds with amino groups, not only within proteins but also lipids and nucleic acids. AGEs can have influence on the function of biological systems by a number of means. Protein modifications can clearly alter structure, enzymatic activity and biological half-life [6,7]. If DNA is altered as a result mutation is occur, and if membrane lipids are altered, this may be effecting transport and signaling processes.

In addition, RAGE directly involved in the development of different diseases and it also affects on oxidative stress and reactive oxygen species (ROS). A reduction in AGEs resulted in decreased disease signs [8,9].

Substantially, the periodontal bacteria promote the flogistic events that lead to an increase in intracellular production of physiological

Reactive Oxygen Species (ROS). Oxidative stress is caused by an unfavorable balance between reactive oxygen species (ROS) and antioxidant defenses. ROS are generated during normal cellular metabolism, as a result of the influence of various environmental factors, as well as during pathological processes [10].

Free radicals are capable of altering all major classes of biomolecules, such as lipids, nucleic acids and proteins, with changes in their structure and function [11]. They mainly target polyunsaturated fatty acids in cell membranes and their interaction results in lipid peroxidation. The levels of free radical molecules are controlled by various cellular defense mechanisms, consisting of enzymatic (catalase, glutathione peroxidase, superoxide dismutase) and non-enzymatic (Vitamin E, Vitamin C and glutathione) components [12].

Oxygen free radicals (OFRs) have been involved in a wide variety of cellular functions but they can be both essential and highly toxic to cellular homoeostasis [13]. OFRs exert their cytotoxic effect by the mechanism of membrane phospholipids peroxidation and it leads to the change in permeability and loss of integrity of membrane [14]. Mammalian cells are equipped with both enzymic and non-enzymic mechanism of antioxidant defenses to reduce the cellular injure caused by contact between cellular constituents and OFRs [13] Reactive oxygen species such as hydrogen peroxide, super oxide and hydroxyl radicals attack biomembranes, and stimulate peroxidation of lipids, leading to an increase impermeability of cell and loss of endothelial integrity. ROS can be produced endogenously or exogenously.

The mitochondria plays very important role and it is a major physiologic source of reactive oxygen species (ROS), which can be generated during mitochondrial respiration. Super oxide radicals, formed by minor side reactions of the mitochondrial electron transport chain or by an NADH-independent enzyme can be converted to H_2O_2 and to a powerful oxidant, the hydroxyl radical. Oxidative stress in organisms leads to the peroxidation of all major biomolecules, such as DNA, proteins and lipids. The most widely used method to find oxidative stress is to determine lipid peroxidation with the Thiobarbituric acid reactive substances (TBARS) method. Among these targets, the peroxidation of lipids is basically damaging because the formation of lipid peroxidation product leads to spread of free radical reactions.

Materials and Methods

Experimental design

This study was conducted according to the rules and regulations of authority. The size of sample is small that's why we first study the lipid peroxidation (TBARS) in tissue was estimated by the method of Ohkawa et al. [15] and then analyze the advance glycation end product (AGE). For the study we divide the study into three groups, First group consist of periimplantitis, second group contain chronic periodontal disease and the third group consist of healthy individuals. The peri-implant tissues were compared with periodontal tissues of healthy and chronic parodontopathic subjects, as suggested by Fritz et al. [16].

Group designing

The study consists of following three groups which are as follows:

Group 1: periimplantitis, using traditional technique which will be failed after six months

Group 2: periodontal disease of chronic form

Group 3: normal healthy individuals for comparing the data

Collection of data

Ten patients of dental implants were selected for study. The implantation was failed within 6 months after implantation. The data consist of 10 subjects (7M/3 F) aged between 40-60 years (average 50.0 ± 4.6). For the preservation of contamination the oral cavity was stored in phosphate buffer saline (PBS), pH 7, 4 (P5368; Sigma–Aldrich, St. Louis, MO, USA) before dry freezing at -80°C.

Tooth collection

Both in the periodontopatic and the healthy group, dental extraction followed the same procedure. After local anesthesia (Mepivacaine 2% and adrenaline 1:100.000 –Scandonest 2% OgnaLaboratoriFarmaceutici - Milan), teeth were extracted and then put in PBS solution before dry freezing at -80°C. We collected 10 teeth from as many subjects with chronic periodontal disease (7 M/3 F) aged between 50-55 years (average 52.2 ± 2.9) and 10 teeth from healthy subjects (7M/3 F) aged between 37-51 years (average 45.0 ± 5.8).

Collection of saliva

The saliva of all enrolled patients was also collected to appraise oxidative stress analysis. Saliva was collected in the morning before any oral operation, after a dynamic washing with water, using Salimetrics® collection system (Salimetrics UK - Oral Swab - Swab Storage Tube) [17].

Processing of tissue sample

The tissue sample was extracted and processed by the method of Takatsu et al. [18]. Both implants and teeth were unfrozen by water bath (bain-marie) at 37°C for 5 min. First, the samples were processed with lancet to remove the small quantity of apical and coronal tissues into the Petri dish with phosphate buffer saline (PBS 1x). The periodontal ligament and the periimplant tissues were so obtained, and then dry frozen at -80°C.

Saliva analysis

The saliva vials were unfrozen and then centrifuged at 6000 rpm for 10 min to obtain whole saliva for analysis of lipid per oxidation. Oxidative stress was measured as lipid peroxidation using specific colorimetric assay for Thio Barbituric Acid Reactive Substances (TBARS).

Analysis of TBARS

TBARS in tissue was estimated by the method of Ohkawa et al. [15]. 0.2 ml tissue homogenate of each group was taken in test tubes and add 200 μl of 8.1% sodium dodecyl sulfate (SDS), Then add 1.5 mL of 20% acetic acid solution (pH 3.5) and 1.5 mL of 0.8% TBA. The mixture was made up to 4.0mL with distilled water and heated in a water bath at 90°C for 60 min. After cooling with tap water, 1.0mL of distilled water and 5.0 mL of n-butanol were added and shaken vigorously and centrifuged at 4000 rpm for 10 minute upper butanol layer was taken and its absorbance at 532 nm was read. The TBARS absorbance, for each sample, was measured three times and the mean value was taken as oxidative stress level, as suggested by the producer.

Analysis of Advance Glycation End product (AGE)

Sample was hydrolyzed 5 times in 0.25 M oxalic acid in boiling water bath, and then dialyzed against phosphate buffered saline (PBS). Protein content of each sample was determined by measurement of the absorption of UV light at 280 nm wavelength and calculated

according to a standard curve. Maillard reaction-related fluorescence (FC), representative of AGEs formed and was measured as an index of advanced glycation in 360/450 nm excitation/emission [19,20] fluorimeter. Quinine sulfate 1 μM in 0.1N H_2SO_4 was used as a standard. The levels of AGEs were expressed as arbitrary fluorescence units (AU) per mg protein.

Results

The statistical analysis for oxidative stress and advanced glycation end product were performed using software SPSS (Version 17.0). The statistical difference in terms of lipid peroxidation (TBARS) and AGEs shows a significant difference between groups. This difference can be explained on the basis of one-way ANOVA with post-hoc Bonferroni correction due to the small sample size. The level of significance was set at $p < 0.05$ for all tests. Results are expressed as mean absorbance value (Table 1 and 2).
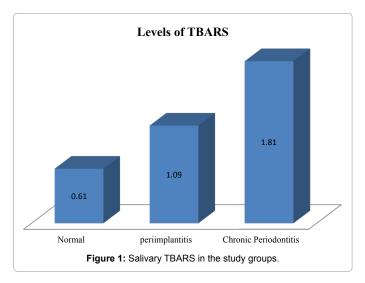
The results showed statistically significant differences in each group. In actual, the chronic periodontal disease group showed higher value of lipid peroxidation than periimplantitis and healthy groups. Periimplantitis group compared to the healthy one had higher oxidative stress levels ($p < 0.001$). Chronic periodontal disease compared to periimplantitis showed higher oxidative stress ($p = 0.002$) as described in Figure 1. Chronic periodontal disease compared to health met the expectation, showing even higher oxidative stress levels than compared to periimplantitis. Analysis showed that AGEs are present in both tissues of periimplantitis and periodontopatic groups compared to healthy subject tissues (Figure 2).
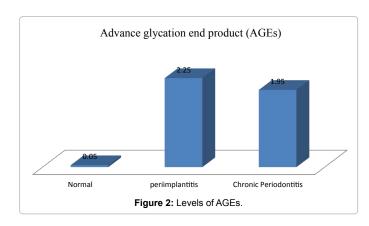
No. of observation	Groups	Mean Absorbance value (μmol/m)
01	Normal	0.61
02	periimplantitis	1.09
03	Chronic Periodontitis	1.81

Table 1: Results of salivary TBARS assay.

No. of observation	Groups	Mean Absorbance value (μmol/m)
01	Normal	0.05
02	periimplantitis	2.25
03	Chronic Periodontitis	1.95

Table 2: Results of Advanced glycation end product (AGE) assay.

Figure 1: Salivary TBARS in the study groups.

Figure 2: Levels of AGEs.

Discussion

In this study we highlighted the role of AGEs and oxidative stress in terms of lipid peroxidation (TBARS) in the progression of periimplantitis and in the failure of dental implants. From our study it is clear that oxidative stress and AGEs production are involved in the failure of dental implants up to some extent. This hypothesis is strongly supported by recent literature, since a possible role of AGEs and oxidative stress was established in many oral inflammatory diseases [8].

This hypothesis is also supported by literature which shows the relationship between oxidative stress and AGEs in the failure of dental implant disease. There are many other factors like smoking, hypertension and poor diet which are involved in the formation of ROS [21]. Many studies from the past literature supported our hypothesis and according to our hypothesis formation of ROs and AGEs involved in the failure of dental implant. The formation of ROS is induced the AGEs which involved in the periimplantitis disease.

Reactive oxygen species (ROS) cause oxidation of DNA, proteins and lipids, and induce carcinogenesis. The major aldehyde products of lipid peroxidation are malondialdehyde (MDA) and 4-hydroxynonenal. MDA is mutagenic in mammalian cells and carcinogenic.

Tetracycline has anti-RAGE activity with antioxidant effects. With the antimicrobial action, these molecules are effective in combating oxidative stress in periodontal disease and have useful effects on systemic diseases motivated by oxidative stress [4].

In addition to drugs therapy, also diet and life style can offer to reduce oxidative stress and AGEs formation. In fact, flavonoids, present in many foods like green tea, chocolate and fruit, have the capacity to reduce glycation [4]. Also vitamins C, E, P have antioxidant proprieties [5]. Therefore, it is clear how dietary factors may play an important role in the strategy against the oxidation.

Conclusion

This introductory study identifies the periimplantitis as a multifactorial disease, in which glycation and lipid peroxidation play a role in terms of etiology and sternness. These finding correlates with the recent literature assumptions that support a closer relationship between systemic conditions and oral health. The molecular pathways that are involved in disease formation unknown, but probably oxidative stress is involved in its etiology, as it is in the periodontitis. These data suggest to the clinician a new strategy to prevent the failure of dental implants. According to our study, periimplantitis as a multifactorial disease, in which glycation and oxidative stress play a role in terms of etiology and severity. This hypothesis could lead to new therapeutic strategies in periimplantitis, using antioxidant approach in addition to conventional treatments.

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