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ORIGINAL RESEARCH

Effect of Titanium Dioxide Nanoparticles on the Activity of Salivary Alkaline Phosphatase in Chronic Periodontitis Patients

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ABSTRACT

Background: Nanotechnology application has rapidly extended into all areas of life. It provides new methods to solve scientific and medical problems. Nanomaterials such as titanium dioxide nanoparticles (TiO_2 NPs) has been used in biomedical applications. Biological influence of the nanoparticles appears to be as a result of the interactions between the nanoparticles and proteins. Salivary alkaline phosphatase (ALP) is an enzyme that is linked to the outer membrane of some cells and formed by many others. ALP activity increase in periodontal diseases especially in the active phase of the disease.

Aim: The aim is to determine the effect of titanium dioxide nanoparticles on the enzymatic activity of alkaline phosphatase in the saliva of patients suffering from chronic periodontitis.

Materials and methods: The influence of titanium dioxide nanoparticles on the enzymatic activity of salivary alkaline phosphatase was examined in 75 participants (44 with chronic periodontitis and 31 nonperiodontitis subjects). The age range of the participants was 35–50 years for both groups. The periodontal disease was determined based on the criteria of periodontal health through examination of plaque and gingival indices together with clinical attachment level and probing pocket depth. Unstimulated saliva was collected from all participants and analyzed.

Results: The results showed that salivary alkaline phosphatase activity was higher in chronic periodontitis patients compare to the nonperiodontitis group and the enzyme activity was found to be not correlated with the periodontal parameters. The enzyme was activated by titanium dioxide nanoparticles.

Conclusion: The effect of titanium dioxide nanoparticles may be attributed to the biological activity of this type of nanoparticles in addition to the conformational changes that can occur on the protein structure after interaction with NPs.

Clinical significance: Recognition of the effect of some nanomaterials (such as titanium dioxide nanoparticles) on enzymes like alkaline phosphatase, may provide a potential therapeutic opportunity for some pathological conditions such as periodontal diseases.

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INTRODUCTION

Chronic periodontitis is a disease of the periodontium that starts as an inflammation of the gingiva and extends into the adjacent bone, and connective tissue.[1] Destruction of tissues associated with periodontal disease is mostly related to the presence of certain pathogenic bacteria in addition to abnormal immune response and The disease has a slow progressive nature but it may exacerbate at any stage causing more tissue destruction and bone loss.[2]

Alkaline phosphatase (ALP) exists as several tissue-specific isozymes. The enzyme is bound to the outside cell plasma membrane and the membrane of matrix vesicles.[3] It is secreted by many organisms ranging from bacteria to man.[4] ALP is a substantial enzyme of the periodontal tissue that has a vital role in the bone turnover process. Increased activity of ALP in saliva may indicate the presence of periodontal disease such as in cases of bone loss caused by periodontitis. Upregulated activity can also be seen in periodontal ligament as a result of tissue renewal.[5-8] *Porphyromonas gingivalis, Prevotella intermedia* and *Capnocytophaga sputigena*. The ALPase activity detected in these bacteria was almost completely inhibited in the presence of 1% sodium dodecyl sulfate (SDS). The mechanism of action of this enzyme has not been fully elucidated. One way to explain this enzyme action is that it increases the concentration of inorganic phosphate, which is considered to be a mineralization promoter, and decreases the concentration of extracellular pyrophosphate, which is considered to be mineralization inhibitor.[3]

Nanotechnology is an emerging technological field with great possibilities to launch great advancements that can be utilized in our day-to-day life.[9] Over the past decade, fast expansion in the nanotechnology field lead to its use in a wide range of application such as drug delivery, imaging and diagnosis.[10-12] Engineering, and medicine with broad

applications for molecular imaging, molecular diagnosis, and targeted therapy. The basic rationale is that nanometer-sized particles, such as semiconductor quantum dots and iron oxide nanocrystals, have optical, magnetic, or structural properties that are not available from molecules or bulk solids. When linked with tumor targeting ligands such as monoclonal antibodies, peptides, or small molecules, these nanoparticles can be used to target tumor antigens (biomarkers). Local drug delivery to specific tissues in periodontal pockets is one of the applications of nanotechnology. It also controls the release of the antibiotic or anti-inflammatory drug into the tissue.[13]

Titanium dioxide (TiO_2) is a scentless, non-inflammable, white powder that exists naturally in some minerals such as anatase, rutile, and brookite. It characterized by high stability, anticorrosive characters, and photocatalysis.[14] Further, TiO_2 NPs have many unique properties such as excellent biocompatibility, chemical stability, and low toxicity; therefore, it has gained huge interest and intensive experimental studies in biomedicine.[15] This study aims to evaluate the action of TiO_2 NPs on the enzymatic activity of salivary ALP in the saliva of a group of patients suffering from chronic periodontitis.

MATERIALS AND METHODS

Sample Selection

This cross-sectional research was performed on 44 individuals have chronic periodontitis and 31 non-periodontitis participants.

Inclusion Criteria

- Periodontitis group with a clinical diagnosis of generalized chronic periodontitis
- Nonperiodontitis group without periodontitis according to their clinical examination.

Exclusion Criteria

- Taking regular medication or antibiotics and/or anti-inflammatory medication that may affect the periodontal tissue or the progression of the periodontal disease
- Systemic diseases
- Smokers
- History of periodontal treatment in the last 6 months
- Pregnancy and lactation. All of them were attending the Dental School Hospital/University of Baghdad. Their age ranged from 35 to 50 years old. Ethical approval for the study was acquired and the consent form was obtained from all participants. All procedures performed in studies involving human participants were in compliance with the 1964 Helsinki Declaration.

Clinical Examination

Includes the examination of:

- Gingival index (GI)[16]
- Plaque index (PLI)[17]
- Clinical attachment level
- Probing pocket depth.

Collection of Saliva

Unstimulated whole saliva samples were collected from all participants in the periodontitis group (group 1) and non-periodontitis group (group 2). The sample was collected between 9 AM and 11 AM, at least one hour after the last meal. Rinsing of the mouth was done before collecting the sample by standard spitting method from both groups. A plain tube was used to gather a total volume of 5 mL for each saliva sample. After collection of the sample was placed in a centrifuge for 15 minutes at 2500 rpm, and then supernatant fluid was gathered and kept in Eppendorf tubes then store at –20° C until its use for chemical analysis.

Laboratory Investigation

Determination of Saliva Sample Volume

Different volumes of salivary sample (20, 40, 60, 80 and 100 µL) were used to determine the optimum saliva volume for calculating the salivary ALP activity for this experiment. It was found that (100 µL) of saliva gives the optimum enzyme activity for this experiment.

Titanium Dioxide Nanoparticles (TiO_2 NPs)

TiO_2 NPs were purchased from Hongwu international group Ltd, Guangdong, China. It is supplied as TiO_2 nanopowder. UV-VIS spectrophotometer (PG Instruments Limited, Lutterworth, United Kingdom) was used to measure absorbance spectra of NPs solution. Measurements were done at room temperature in a quartz cell with an optical path of 1 cm. Transmission electron Microscope TEM (Philips Electron Optics, Eindhoven, Netherlands) was used to identify the form and the size of the nanoparticles sample.

Salivary ALP Enzyme Assay

A spectrophotometric method was used to calculate the salivary ALP activity according to the recommendation of the manufacturer instruction using ALP detection kit (colorimetric) provided by Human Company, Wiesbaden, Germany. The reaction mix consist of a substrate:

- 1.25 mol/L of diethanolamine buffer (pH 10.35 ± 0.2)
- 50 mmol/L of p-Nitrophenylphosphate
- 0.625 mmol/L of magnesium chloride, p-nitrophenol is formed as a result of p-nitrophenyl phosphate

reduction in the presence of a salivary ALP enzyme. The increase in ALP enzyme activity is directly proportionate with an increase absorbance at 405 nm.

TiO_2 Nanoparticles Solution Preparation

A solution of (300 µg/mL) of TiO_2 NPs was made and then it was diluted by using a solvent (3:1 water: ethanol) to (20, 40, 60, 80, 100 µg/mL)concentrations to determine the best concentration for this experiment.

Determination of the Effect of TiO_2 NPs on the Activity of Salivary ALP

The activity of ALP enzyme was measured by adding 20 µL of TiO_2 NPs solution to 100 µL of saliva and then using the detection kit according to the instruction. The spectrophotometer was used to measure the absorbance of light at a wavelength of 405 nm and determine the activity of salivary ALP in the sample. By comparing the activity of the enzyme before and after the addition of the nanoparticles, the percentage of the effect on the enzyme activity was measured according to the following formula:

activation percentage = 100 – 100 × (Activity of the enzyme with TiO_2 NPs /Activity of enzyme without TiO_2 NPs)

RESULTS

Clinical Findings

The mean score of both PLI and GI in the periodontitis group (group 1) were highly significant compared to the non-periodontitis group (group 2) ($p < 0.0001$). The mean ± standard deviations (SD) of the clinical parameters PLI and GI for both groups are shown in Table 1.

In group 1 the mean±SD of PPD were (3.92 ± 0.88), and for CAL were (4.38 ± 0.86) as stated in Table 2.

TiO_2 NPs Characterization

Absorbance feature of TiO_2 NPs was illustrated in Figure 1. The peak can be identified. The peak, which represents the absorbance intensity of TiO2 NPs dispersion in the UV region at less than 300 nm, was found to be around 200 nm.

The form and size of the nanoparticles samples were specified by the transmission electron microscope TEM (Fig. 2). The particles size were calculated and found to have an average diameter of <30 µm.

Table 1: Mean plaque and gingival index for group 1 (n = 44) and group 2 (n = 31)

Periodontal parameters	*Groups*	*Mean ± SD*	*p value*
PLI	1	1.73 ± 0.30	0.0001*
	2	1.03 ± 0.04	
GI	1	1.50 ± 0.35	0.0001
	2	1.03 ± 0.06	

*Significant *p* value <0.0001

Biochemical Analyses

Intergroup comparison of the activity of ALP in saliva samples revealed a significantly higher enzymatic activity in group 1 compared to group 2 ($p < 0.001$) (Table 3).

In both groups, there was a non-significant correlation between ALP activity and clinical periodontal parameters of PI and GI (Table 4).

Different concentrations of TiO_2 NPs (20, 40, 60, 80, 100) were used to determine the best nanoparticles concentration for this experiment (Fig. 3). Optimum enzyme activity was recorded at 100 µg/mL of TiO_2 NPs. The real concentration of the TiO_2 NPs in the total volume of the reaction mixture (1370 µL) was then calculated. The highest effect of TiO_2 NPs on enzyme activity was found to be at concentration of 1.4 µl/mL in a total volume of the reaction mixture.

In an attempt to comprehend the potential influence of TiO_2 NPs on the enzymatic activity of salivary ALP in patients having chronic periodontitis, the activity of the enzyme was measured before and after the addition of the NPs in both groups 1 and 2. An intragroup comparison of the activity of salivary ALP enzyme in group 1 before and after the addition of TiO_2 NPs revealed a high statistical significance ($p < 0.0001$). A high statistical significance was also found when comparing between the enzyme activity in group 2 before and after the addition of TiO_2 NPs ($p < 0.0001$). The results are shown in Table 5.

Intergroup comparison revealed a statistically significant changes in the activity of salivary ALP when adding TiO_2 NPs in both groups 1 and 2 (p value < 0.001) (Table 6).

Table 2: Mean and SD of PPD and CAL in the periodontitis group (group 1, n = 44)

Periodontal parameters	*Mean± SD*
PPD	3.92 ± 0.88
CAL	4.38 ± 0.86

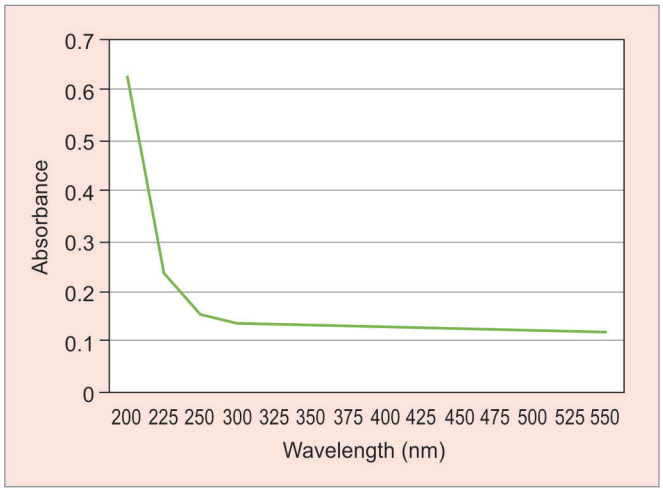
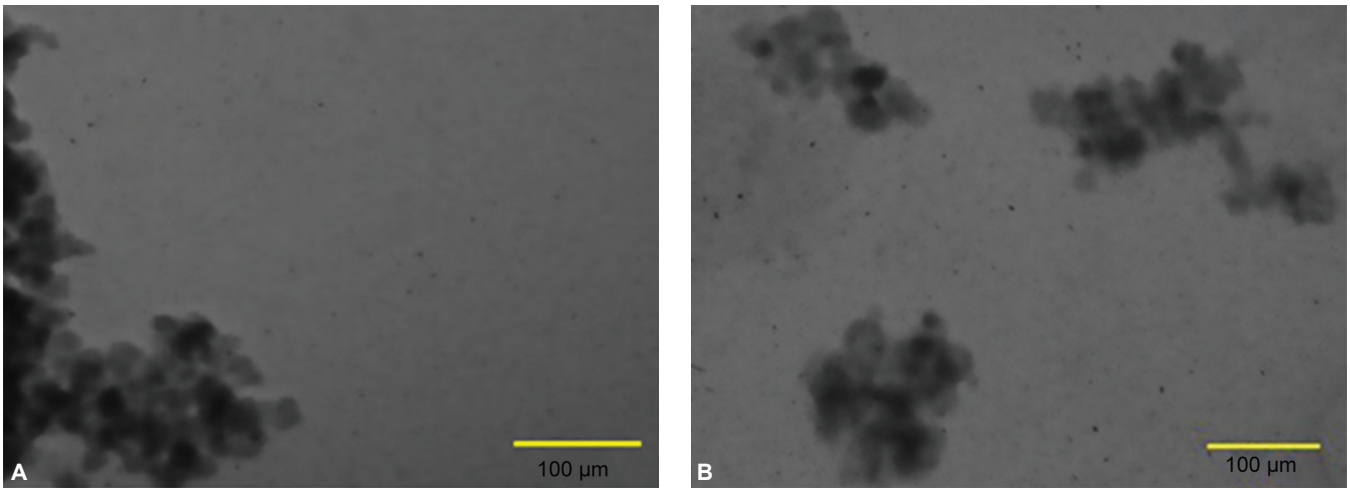


Fig. 1: Absorbance spectra of the TiO_2 NPs



Figs 2A and B: Titanium dioxide nanoparticles under the transmission electron microscope

Table 3: Mean ALP activity and statistically significant for group 1 (n = 44) and group 2 (n = 31)

Group	*Mean ± SD*	*p value*
1	0.8 8 ± 0.6	0.0001*
2	0.4 1 ± 0.32	

*Statistically significant difference between groups 1 and 2 (student t-test), *p* value 0.0001

Table 4: Correlation and *p* value between salivary ALP activity and clinical periodontal parameters (PL and GI) in both groups

Groups	*Periodontal parameters*	*r**	*p value*	
1	PLI	0.13	0.36	NS**
	GI	0.09	0.54	NS
2	PLI	0.02	0.87	NS
	GI	0.13	0.5	NS

*Coefficient of correlation
**Nonsignificant *p* value ≥0.05

Table 5: Salivary ALP activity before and after the addition of TiO_2 NPs in both groups

Groups	*Salivary ALP activity in U/L Mean ± SD*	*p value*
1 (with TiO_2 NPs)	1.51 ± 1.07	0.0001*
1 (without TiO_2 NPs)	0.88 ± 0.6	
2 (with TiO_2 NPs)	0.89 ± 0.48	0.0001*
2 (without TiO_2 NPs)	0.41 ± 0.32	

*Significant *p* value <0.0001

Statistical Analysis

It was performed using the statistical package for social science (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp) and Microsoft Office Excel 2010. Parametric statistical methods were used to analyze the clinical and enzymatic data. The statistical significance was determined using the student t-test ($p < 0.05$). Pearson correlation coefficient was also used. For PLI and GI data, the mean value for each patient was calculated, and afterward, a calculation of the mean value for each group was done. For analysis of the activity of ALP enzyme in saliva, the value of enzymatic activity with the addition of nanoparticles and enzymatic activity without the addition of the nanoparticles for each group was obtained.

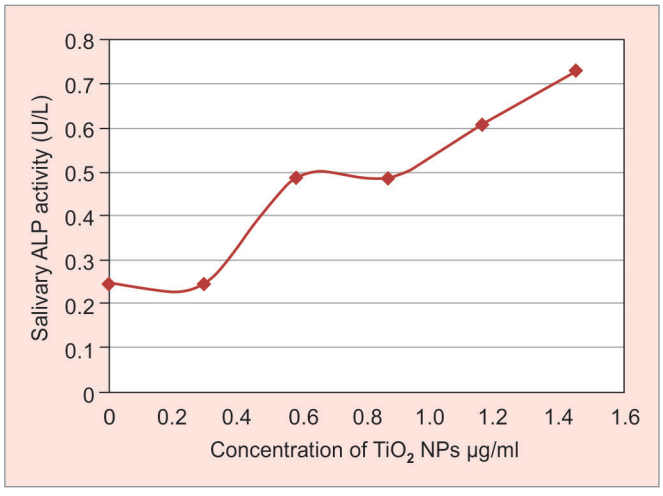


Fig. 3: Effect of different concentrations of TiO_2 NPs on salivary ALP activity

Table 6: Comparison between salivary ALP activity in groups1 and 2 with TiO_2 NPs

Group	*Salivary ALP activity in U/L Mean ± SD*	*p value*
Group 1 (with TiO_2 NPs)	1.51 ± 1.07	0.001 *
Group 2 (with TiO_2 NPs)	0.89 ± 0.48	

*Significant *p* value <0.001

DISCUSSION

The focus of this study was to evaluate the effect of TiO_2 NPs on the enzymatic activity of salivary ALP in chronic periodontitis patients. According to our knowledge, no published report on the action of this nanoparticleson the activity of salivary ALP in chronic periodontitis patients was found, so results of this study could not be compared with other studies.

The chronic periodontitis patients presented significantly higher ALP activity than nonperiodontitis

patients. These results agree with the results of other studies that revealed an increase of activity of salivary ALP in patients with chronic periodontitis compared to gingivitis or healthy individuals.[18-20] This can be explained as due to the release of the enzyme by bacterial cells and inflammatory cells.[21] ALP is released by secondary granules of neutrophils and its concentration increases greatly with an increase in inflammation.[22] This enzyme is released from dead and dying cells of the periodontium, mostly from polymorphonuclear leukocytes. ALP is also produced by cells like fibroblasts, osteoblasts, and osteoclasts. During the active stage of periodontitis, the cell membrane of these cells will be ruptured releasing the intracellular contents outside which lead to increase secretion of ALP in the gingival crevicular fluid and saliva of periodontitis patient.[23-26]

In both groups, the study showed no significant correlation between salivary ALP and clinical periodontal parameters of GI and PLI. These results agree with results from a study on the activity of salivary ALP in individuals suffering from chronic periodontitis in which it revealed the absence of any correlation between this enzyme activity and periodontal parameters.[27] A different study revealed no significant correlation in clinical parameters and salivary parameters.[28] In contrast, in a study the activity of the enzyme was found to be positively correlated with periodontal parameters.[29] A study by Todorovic et al.[30] showed a between the values of the GI activity of some salivary enzymes such as ALP. This can be attributed to the difference in sample size, a method of collecting saliva, and method of analysis.

The biochemical tests showed that TiO_2 NPs caused an increase in salivary ALP activity (activation effect). In another similar study, to determine the effect of TiO_2 nanoparticles on the activity of salivary ALP enzyme in individuals with gingivitis, it was found that the TiO_2 NPs caused inhibition of the salivary ALP activity at lower concentrations more than the higher range of concentrations.[18] In kinetic study on the action of TiO_2 NPs on the enzymatic activity of salivary peroxidase, salivary peroxidase was found to be activated by TiO_2 NPs.[31] A study on the effect of zinc oxide NPs (ZnO NPs) on the enzymatic activity of salivary ALP in chronic periodontitis patients showed that ZnO NPs caused an increase in the salivary ALP activity.[32] Another study on the effect (ZnO) NPs on ALP activity in mouse myoblasts cells, showed that increase ZnO NPs concentration caused a significant increase in ALP activity in a dose-dependent manner.[33] While In a recent study by Ghudhaib et al.,[34] on aspartate aminotransferase (AST) enzyme it was shown that the activity of salivary AST in presence of ZnO NPs in chronic periodontitis group was higher than its activity in the group without ZnO NPs. Another recent study on the effect of ZnO NPs on the activity of salivary ALT in individuals with chronic periodontitis showed an activation effect of this nanoparticles on salivary ALT.[35] The increase in the activity of salivary ALP after the addition of TiO_2 NPs can be attributed to the biological activity of this type of NPs in addition to the conformational changes that can occur on the protein structure after interaction with NPs and this may cause rise in the accessibility of a substrate to the active site of its specific enzyme. The structure of the protein can be modified when they interact with the surface of the NPs. This structural modification can cause a change in the conjugated protein biological function.[36]

The limitation of this study was the small size of the sample because of the exclusion criteria. Although significant results were obtained in this study and TiO_2 NPs showed promising results in promoting the enzyme activity. Further researches should be conducted on larger sample size and using site-specific methods for more precise results.

CONCLUSION

The results in this study showed increase in ALP activity in presence of TiO_2 NPs. In saliva of chronic periodontitis patients, it was found that the ALP enzyme level was significantly higher than the nonperiodontitis group.

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