Research Article

The Effect of titanium dioxide nanoparticles on the activity of salivary peroxidase in periodontitis patients

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Abstract: Background:The technology of nanoparticles has been expanded to many aspects of modern life. Titanium dioxide nanoparticles were of many nanomaterials utilized in biomedical applications. The interactions between nanoparticles and proteins are believed to be the base for the biological effect of the nanoparticles. The oxidation reaction of many substances is catalyzed by oxidizing enzymes called peroxidases. The activity of salivary peroxidase is elevated with periodontal diseases. the aim ofthis study is to examine the action of titanium dioxide nanoparticles on salivary peroxidase activity.Material and method75 participants were enrolled in this study—Periodontitis group with 44 participants and the non-periodontitis group with 31 participants. The participants' age range was 35 to 50 years for both groups. The clinical parameters of plaque index, gingival index, probing pocket depth and clinical attachment level were used in this study to determine the presence or absence of the periodontal disease. Unstimulated saliva was collected from all participants and analyzed for the activity of peroxidase enzyme under the effect of titanium dioxide nanoparticles. ResultsThe Periodontitis group showed higher peroxidase enzyme activity than the non-periodontitis group and the activity of salivary peroxidase showed no correlation with the clinical parameters. Titanium dioxide nanoparticles increased salivary peroxidase activity. ConclusionThis study demonstrated that the solid surface of nanoparticles could induce changes in the attached protein molecule which in turn causes changes in the effect of the nanoparticles on living tissue or organism. The titanium dioxide nanoparticles Play a role in increasing the activity of salivary peroxidase within the saliva of chronic periodontitis patients.

Keywords: *TiO2 nanoparticles, salivary peroxidase, periodontitis, saliva.*

Introduction

Periodontitis is a wide-spreading disease characterized by the pain-free and sluggish spread of the condition. This disease starts with a plaque under the gingiva and is altered by the immune response. The disease can occur in different age groups but is most common in adults. It causes loss of the periodontium, which in time leads to tooth mobility and loss [1,2].

Periodontitis and other inflammatory diseases are related to what is known as oxidative stress. In periodontitis, an imbalance or a shift occurs between antioxidant and oxidant enzymatic and non-enzymatic defense systems. During inflammation, the polymorph nuclear leukocytes make reactive oxygen species (ROS) which are very disastrous to the tissue. The damage caused by these ROS is removed or repaired by antioxidants (AO)[3].

Peroxidases are oxidizing enzymes that trigger oxidation reactions for different materials. Hydrogen peroxidase (H2O2) helps the reaction to continue then it's reduced to water [4]. Peroxidase can be found in

bodily fluids such as saliva and tears. In addition, it can be found in cells, removing free radicals with the assistance of H2O2. The two structures of peroxidases, which are salivary peroxidase and myeloperoxidase, are detected in the whole saliva and play an important role in the defense mechanism [5].

Nanomaterials consist of components less than 100 nm in at least one dimension[6]. Titanium dioxide nanoparticles (TiO2 NPs) have many distinctive qualities and properties such as compatibility with living tissue and optical properties[7] and many studies showed its antibacterial activity[8,9] when added to dental materials, Therefore TiO2 NPs have been investigated profusely in recent years to make advances in dentistry. te aim of this study was to analyze the action of TiO2 NPs on peroxidase enzyme activity in patients suffering from chronic periodontitis.

Subjects and Methods

1. Sample selection:

In this study, two study groups were recruited. The first group (group 1) consisted of 44 participants (periodontitis group), while the second group (group 2) consisted of 31 participants (non-periodontitis group). The total number of participants was 75 and the age range was from 35 to 50 years. The collection of the samples started in October and finished in December of 2017. The participants were patients seeking treatment in the Department of Periodontics at the College of Dentistry, University of Baghdad. Both consent forms and ethical approval were acquired for this study.

2. Clinical examination:

The periodontal parameters (gingival index[10], plaque index [11], bleeding on probing, clinical attachment level, and probing pocket depth were used for the assessment and diagnosis of the periodontal condition of the participants.

3. Collection of Saliva

The spitting method for the collection of whole unstimulated saliva was used. Patients were asked to rinse their mouth first, then wait a few minutes before spitting into a plane tube to collect 5 ml of saliva. This was done at least one hour after the participant's last meal. The time of sample collection was between 9 a.m.- 11 a.m. After the collection of the sample; it was centrifuged for 15 minutes at 2500 rpm. A layer called supernatant is formed and then collected and stored in an Eppendorf tube at -20˚C.

4. Laboratory procedures:

a. Saliva sample volume determination:

To determine the best saliva volume to obtain the optimum activity of peroxidase, different volumes of saliva samples were tested (20, 40, 60, 80, and 100µl).

In this experiment, different volumes of saliva were collected (20, 40, 60, 80 and 100 µl) to determine the best saliva volume to measure the activity of salivary peroxidase. The optimum enzyme activity for this experiment was found to be (100 µl).

b. Characterization of Titanium dioxide nanoparticles:

TiO2 Nanopowder was obtained from Hongwu international group Ltd, Guangdong, China. An ultraviolet-visible spectrophotometer (PG Instruments Limited/ United Kingdom) was used to determine the absorption spectra for the TiO2 NPs solution used in this study. The measurement was done at room temperature. The size and structure of the Tio2 NPs in the samples were determined using a transmission electron microscope TEM (Philips CM10).

c. Salivary Peroxidase Assay:

The colorimetric method was used to determine the activity of the peroxidase enzyme in saliva. In this study, 4-aminoantipyrine was used as a hydrogen donor. The H2O2 decomposes through the incubation period causing an elevation in absorption at λ= 510 nm, which helps in determining the activity of the enzyme, after adding 1.4 ml of 4-aminoantipyrine (2.5 mM) with phenol (0.17 M) solution to 1.5 ml of H2O2 (1.7 mM) in a buffer of phosphate (0.2 M) of pH 7.0 solution. The initiation of the reaction was done by adding saliva (100µl). To obtain (ΔA/min) the elevation in absorbance at 510 nm was calculated for 5 minutes. One unit of enzyme activity represents one µmole decomposition of H2O2 through the period of one minute at pH =7.0 under certain conditions.

d. Preparation of TiO2 NPs solution:

The first step was to prepare a stock solution of TiO2 NPs (300 µg/ml). A solvent of 3:1 water to ethanol was used to dilute the stock solution to different concentrations (20, 40, 60, 80, 100, and 120 µg\ml). The best concentration was found to be 120 µg\ml.

e. Determination of the action of TiO2 NPs on the activity of peroxidase enzyme:

A detection kit was used to measure the action of TiO2 NPs on the activity of peroxidase enzyme by adding 20 µl of 120 µg\ml TiO2 NPs to 100 µl of saliva and then using the detection kit. To measure the absorbance at a wavelength of 510 nm and establish the peroxidase activity in the sample. A percentage of activation equation was used to calculate the effect percentage.The activity of the enzyme with the presence of TiO2 NPs and without the nanoparticles was compared according to the percentage of activation equation to calculate the percentage of effect on the peroxidase enzyme activity:

% activation = 100 – 100 × [Activity in the presence of nanoparticles/Activity without the nanoparticles].

5. Statistical analysis:

Statistical package for social sciences (SPSS) and Microsoft Office Excel were used to analyze the collected data. The significant difference was assesedusing the student t-test ($P < 0.05$). The Pearson correlation coefficient was also used.

Results

1. Clinical findings

The mean values and standard deviations (SD) of Peroxidase activity for group 1 were highly significant compared to group 2 ($p< 0.001$).

The mean values and SD of Peroxidase activity for both groups are shown in table1:

Table 1: mean, standard division, t-test and p-value of Peroxidase activity for group1 and group2:

Groups	N	Mean ±SD	t-test	p-value
Group1	44	242.38±137.31	2.68	0.001^{S}
Group2	31	175.67±76.08		

N: Number, SD: Standard Deviation, t-test: student's t-test, p-value: Probability value, S: significant p-value ($p< 0.001$)

The correlation was non-significant when comparing the activity of peroxidase and both plaque index (PLI) and gingival index (GI) in group 1. A weak negative and non-significant correlation was determined for GI and a non-significant correlation for PLI in group 2. Results are as illustrated in the table below:

Table 2: correlation and p-value between periodontal parameters and peroxidase activity in group1 and group 2:

Periodontal parameters	Study groups			
	Group1, N=44		Group 2, N=31	
	r	p_value	r	p_value
PLI	0.044	0.777 ^{NS}	0.360	0.05 ^{NS}
GI	0.261	0.086 ^{NS}	-0.011	0.951 ^{NS}

r: Coefficient of correlation, p_value: Probability value, PL: Plaque index, GI: Gingival index, NS: Non – Significant p_value $\geqslant$ 0.05, N: Number.

2. TiO2 NPS characterization:

Spectra of UV-VIS absorption were used to determine TiO2 NPs absorption qualities. The peak was around 200 nm which points to the intensity of absorption of TiO2 NPs dispersion at <300 nm in the ultraviolet area of the spectrum, as shown in Figure (1):

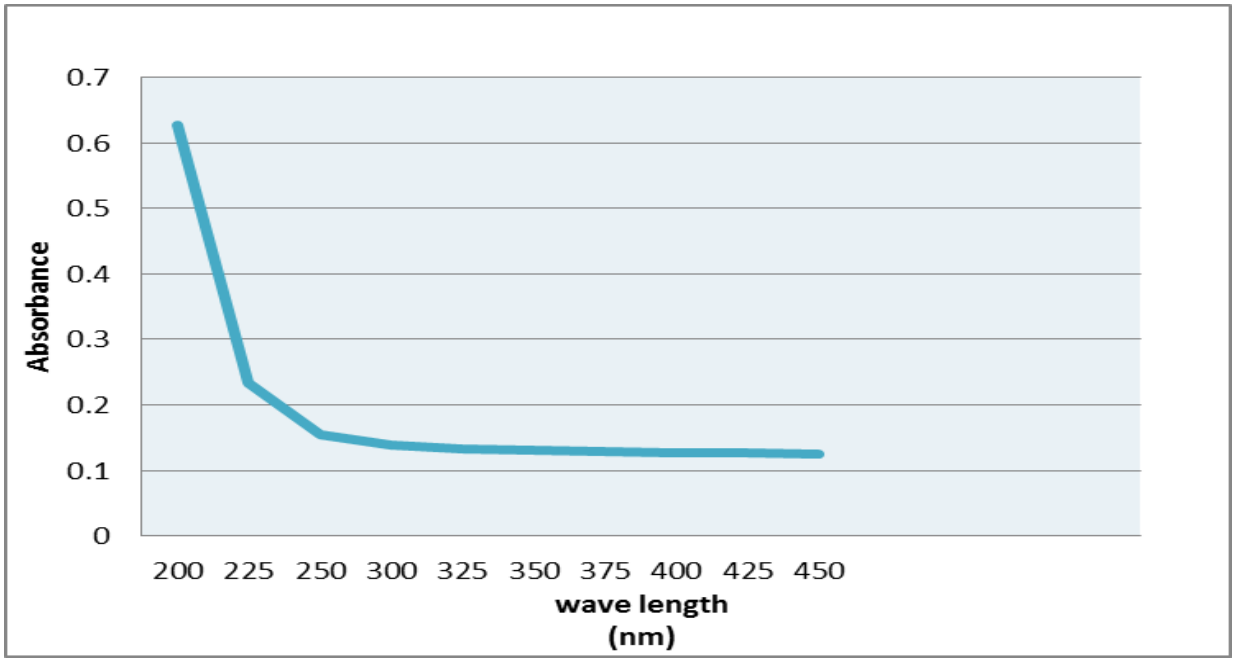

Figure 1: Absorbance spectra of TiO2 nanoparticles.

TEM was used to identify the structure and Nano size measurement of TiO2 NPs in the samples (figure 2). The average diameter of the particle size was found to be < 30nm.

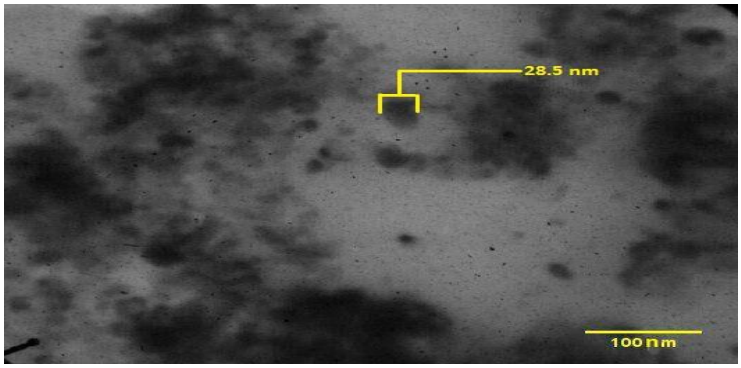

Figure 2: The TEM pictures and size distribution of TiO2 NPs. The arrow shows a nanoparticle size of 28.5nm (less than 30 nm).

3. The effect of TiO2 NPs on Peroxidase enzyme in saliva

The differences in the activities of peroxidase enzyme (mean ± SD) for the studied groups are shown in Table (3) below. The results conveyed that the activity of peroxidase in saliva samples from group 1 with TiO2 NPs was greater than the enzyme activity in samples without TiO2 NPs with a statistically high significance difference (p-value< 0.001), as shown in the table below. Similarly, a highly significant difference was also found After comparing the enzyme activity in group 2 with and without TiO2 NPs.

Table 3: intragroup comparison of the effect of TiO2 NPs on peroxidase activity in both groups.

	Study groups					
	Group1, N=44			Group1, N=31		
	MEAN± SD	t-test	P_value	MEAN± SD	t-test	P_value
with TiO2 NPs	304.81± 153.43	7.230	0.0001HS	213.48± 88.65	5.036	0.0001HS
without TiO2 NPs	242.38± 137.31			175.67± 76.08		

TiO2 NPs: Titanium Dioxide Nanoparticles, HS: Highly Significant (p_value< 0.001)

The activity of peroxidase enzyme in the presence of TiO2 NPs was compared between both groups. The results showed a high statistically significant difference (p-value< 0.001), as stated in Table (4) below:

Table 4: Intergroup comparison of the effect of TiO2 NPs on peroxidase activity in both groups and significance level:

Groups	peroxidase Activity in U/L Mean ±SD	t-test	P_value
Group1(With TiO2 NPs)	304.81± 153.43	3.252	0.001HS
Group2 (With TiO2 NPs)	213.48± 88.65		

Figure (3) shows the action of TiO2 NPs (µg/ml) on the peroxidase activity (U/L) in the reaction mix of a total volume of (3020). It was established that TiO2 NPs at a concentration of 0.79 µg/ml have a higher activation effect on the enzyme activity in the mixture.

At a concentration of 0.79 µg/ml of TiO2 NPs, peroxidase activity reached its highest activation percentage (65.93%), making this concentration of the nanoparticles the most effective concentration in this experiment as shown in Figure (4).

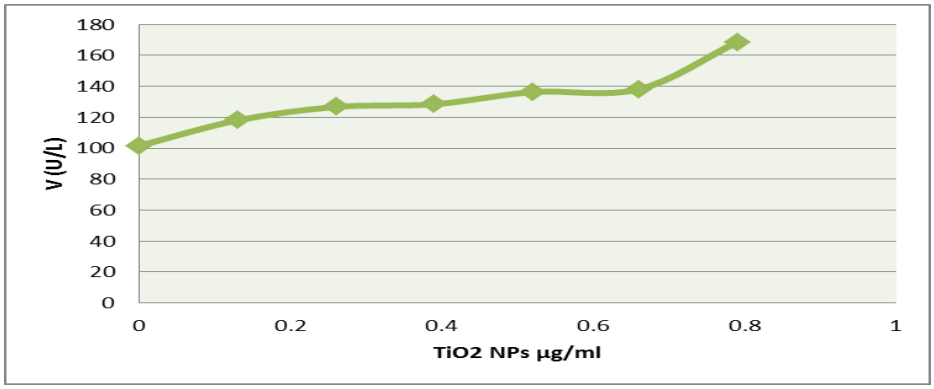

Figure 3: effect of different concentrations of TiO2 NPs on salivary peroxidase activity.

V= volume, U/L= units per liter, µg/ml= microgram per millilitre, TiO2 NPs= titanium dioxide nanoparticles

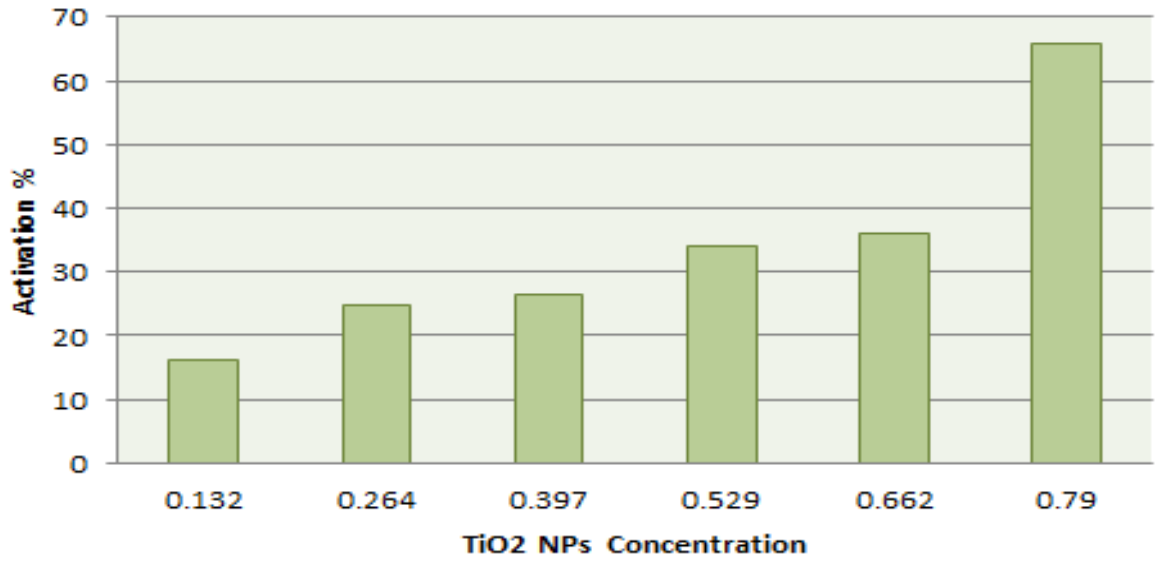

Figure 4: Percentage of activation of salivary peroxidase activity in different concentrations of TiO2 NPs. TiO2 NPs= titanium dioxide nanoparticles

Discussion

Nowadays, TiO2 NPs were incorporated in many products used in daily life which made it necessary to evaluate the action of this type of nanoparticle on living systems[12].

The chemical evaluation revealed that TiO2 NPs caused an elevation in peroxidase activity. Al-Rubaee et al. in 2016 showed that Total salivary peroxidase was activated significantly ($p<0.001$) by TiO2 NPs[13]. The surface of the nanoparticles can cause changes in the form of the protein when it attaches itself to the nanoparticle surface. This change in the protein form can lead to a change in the protein's function. This changes the bio reactivity of the nanoparticles[14].

Many studies showed evidence of conformational changes when enzyme interacts with NPs, such as a study on Zinc oxide NPs in which it was found that the NPs modify the secondary structure of lysozyme. The enzyme keeps its catalytic activity and resists denaturation in the presence of these NPs[15].

In another study to determine the effect of zinc oxide nanoparticles on peroxidase enzyme activity, an inhibition action on peroxidase enzyme activity was noticed for this type of nanoparticles[16]. The activity of peroxidase was found to be elevated in periodontitis compared to non-periodontitis patients, with a significant difference. Similar results were obtained in another study by Al-Rassam et al. in 2017 [17] in which a comparison was made between salivary peroxidase activity in the chronic periodontitis group and a control group, the enzyme activity was elevated in the chronic periodontitis group compared to the control group and with a significant difference. Another study found that salivary peroxidase activity significantly increases with inflammation and reduces after oral hygiene measures[18].

In another study, Glutathione peroxidase in saliva and gingival tissues of subjects with and without chronic periodontitis was evaluated and it was found that there was an increase in Glutathione peroxidase level in saliva and inflamed gingival tissue[19].

The elevated peroxidase level in the saliva and tissue of the gingiva of patients suffering from periodontitis can be accredited to the scavenging of the redundant lipid peroxidation products at the inflammatory sites[20].

Also, the elevated level of reactive oxygen species (ROS) formed may have caused oxidative stress, which lead to an increased need for peroxidase generation to establish the ROS–AO balance to prevent tissue damage[19].

In this study, a non-significant correlation between the gingival index and the activity of salivary peroxidase in group1 and group2, a similarly non-significant correlation was found between plaque index and the action of salivary peroxidase in both groups (P> 0.05). This is, in contrast, to a study by Dagar et al. in 2015 [21] in which a significant correlation was found between the activity of peroxidase and PLI. This difference in results could be accredited to the variation in saliva-collecting methods, the number of participants in each study, and analysis methods.

In this study, TiO2 NPs caused an increase in the action of the peroxides enzyme. Peroxidase enzyme level in the saliva of patients suffering from periodontitis was significantly higher than patients without periodontitis disease.

However, more research are needed to overcome the limitations of this study. Some of these limitations, such as the limited study sample size, the time constraints, and the technique-sensitive method for preparing the nanoparticle solution, can be addressed in future studies for more precise results.

Conclusion

The solid surface of nanoparticles can induce changes in the attached protein molecule which in turn causes changes in the effect of the nanoparticles on living tissue. The titanium dioxide nanoparticles play a role in increasing the activity of salivary peroxidase within the saliva of periodontitis patients.

Conflict of interest: None

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تاثير جزيئات ثنائي اوكسيد التيتانيوم النانوية على فعالية انزيم البيروكسيديس في لعاب المرضى المصابين بالتهاب دواعم السن

الباحثون: طبيب الاسنان الاختصاص ميس علاء ,أستاذ باسمة غفوري ,أستاذ دكتور ايمان الربيعي وطبيب الاسنان الاختصاص ميساء مهدي

الخلاصة

الخلفية: لقد تم توسيع تقنية الجسيمات النانوية لتشمل العديد من جوانب الحياة الحديثة. كانت الجسيمات النانوية لثاني أكسيد التيتانيوم واحدة من العديد من المواد النانوية التي تم استخدامها في التطبيقات الطبية الحيوية. يعتقد أن التفاعلات بين الجسيمات النانوية والبروتينات هي أساس التأثير البيولوجي للجسيمات النانوية. يتم تحفيز تفاعل الأكسدة للعديد من المواد عن طريق إنزيمات مؤكسدة تسمى البيروكسيداز. يرتفع نشاط البيروكسيداز اللعابي في أمراض اللثة.لدراسة تأثير الجسيمات النانوية لثاني أكسيد التيتانيوم على نشاط بيروكسيداز اللعاب ، تم تسجيل 75 مشاركًا في هذه الدراسة. مجموعة التهاب دواعم السن 44 مشاركًا ومجموعة غير التهاب دواعم السن مع 31 مشاركًا. تراوحت أعمار المشاركين من 35 إلى 50 عامًا لكلا المجموعتين. تم استخدام المعلمات السريرية لمؤشر اللويحة ، مؤشر اللثة ، فحص عمق الجيب ومستوى الارتباط السريري في هذه الدراسة لتحديد وجود أو عدم وجود مرض اللثة. تم جمع اللعاب غير المحفز من جميع المشاركين وتحليل نشاط إنزيم البيروكسيديز تحت تأثير الجسيمات النانوية لثاني أكسيد التيتانيوم. أظهرت مجموعة التهاب دواعم السن زيادة نشاط إنزيم البيروكسيديز مقارنة بمجموعة غير التهاب دواعم السن ، ولم يظهر نشاط البيروكسيداز اللعابي أي ارتباط بالمعايير السريرية. تم زيادة نشاط بيروكسيداز اللعاب بواسطة جزيئات ثاني أكسيد التيتانيوم النانوية. ظهرت هذه الدراسة انه بالامكان أن يتسبب السطح الصلب للجسيمات النانوية في إحداث تغييرات في جزيء البروتين المرتبط والذي يؤدي بدوره إلى تغيرات في تأثير الجسيمات النانوية على الأنسجة الحية أو الكائن الحي. يلعب **TiO2 NPs** دورًا في زيادة نشاط بيروكسيديز اللعاب داخل لعاب مرضى التهاب دواعم السن المزمن.