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**Research Article**

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**THE EFFECT OF TITANIUM DIOXIDE NANOPARTICLES ON SALIVARY ALKALINE PHOSPHATASE ACTIVITY**

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**ABSTRACT**

The structural and optical properties of the titanium dioxide nanoparticles (TiO<sub>2</sub>NPs) have been investigated using (UV-Vis) spectrophotometer and SEM. The produced nanoparticles show small and about sharp round peaks around 220nm. The produced nanoparticles have a spherical shape with an average particle size 50 nm. The effect of titanium dioxide NPs was studied on the activity of Alkaline Phosphatase (ALP) in the saliva of 25 patients with gingivitis in comparison to 20 healthy subjects with the average age about 22-23 years for both groups. The results correlated with the observation that salivary alkaline phosphatase activity increase in patient with gingivitis in comparison to control group and salivary ALP activity inhibited by titanium dioxide nanoparticles.

**Keywords:** TiO<sub>2</sub> nanoparticles, ALP activity, Gingivitis, Saliva.

**INTRODUCTION**

Periodontal diseases are bacterial infections of the gingiva, bone and attachment fibers that support the teeth and hold them in the jaw. The main cause of the diseases is a bacterial plaque, a sticky, colorless microbial film that constantly forms on teeth<sup>(1)</sup>. Microbial plaque accumulation on teeth surfaces adjacent to the gingival tissues brings the oral sulcular and junctional epithelial cells into contact with the waste product, enzymes and surface components of colonizing bacteria. As the bacterial load increases so do the irritation of the host tissues by these substances<sup>(2)</sup>. From the periodontal diseases gingivitis and periodontitis are the two predominant periodontal diseases and may be present at the same time. In gingivitis, the gingiva are inflamed but their destruction is reversible while in periodontitis the inflammatory changes

are irreversible changes<sup>(3)</sup>. Gingivitis represents the inflammation process limited to the gingival tissue next to the tooth without any loss of attachment and possibly acts as a precursor to attachment loss around the tooth<sup>(4)</sup>. Gingivitis is typically characterized as a vigorous inflammatory response limited mostly to the superficial gingival connective tissues and is a comparatively nonspecific response to a nonspecific bud up of dental plaque. With continuing plaque accrual, gingivitis becomes well established, but still confined to the superficial gingival connective tissues<sup>(5)</sup>.

In humans, alkaline phosphatase (ALP) is existing in each tissue through the entire body, but is specially concentrated in liver, bile duct, bone, kidney, the placenta and intestinal mucosa<sup>(6)</sup>. The enzyme ALP plays a role in bone metabolism. It is glycoprotein enzyme that bounded to membrane and it

produced by many cells, such as leukocytes, polymorphonuclear, macrophages, fibroblasts, and osteoblasts in the area of the gingival tissue and periodontium<sup>(7)</sup>. Gao J et al.<sup>(8)</sup> show that the highest ALP activity was found in osteoblasts, but moderate in periodontal ligament PDL fibroblasts, and lowest in gingival fibroblasts. No activity was detected in cementoblasts. In the periodontium, ALP is very important enzyme as it is part of normal turnover of periodontal ligament, root cementum and maintenance, and bone homeostasis. Some forms of enzyme are also produced by plaque bacteria<sup>(9)</sup>. Some studies have shown a strongly increased activity of salivary alkaline phosphatase in periodontal disease particularly in the acute phase, and after the periodontal rehabilitation, the activity of these enzymes restored to the normal value that found in the healthy persons<sup>(10)</sup>.

Nanoparticles have many different effects on human health relative to bulk original material from which they are produced<sup>(11)</sup>. Consequently of their tiny size, nanoparticles may propose other advantages to the biomedical field through increased biocompatibility<sup>(12)</sup>. Studies of the effects of nanoparticles (NPs) from different industry branches on cells and pathways are emerging, and most of the biological effects of NPs seem due to their interactions with proteins<sup>(13)</sup>. Enzymes are biologic polymers that catalyze the chemical reactions that make life possible, with the exception of catalytic RNA molecules, or ribozymes, enzymes are proteins<sup>(14)</sup>.

In current years, metal oxide nanoparticles have been mainly examined for their antimicrobial activity, especially titanium dioxide which is considered a clean photo catalyst, low-cost, with nontoxicity and chemical stability<sup>(15, 16)</sup>. Titanium dioxide nanoparticles are produced universal for multiplicity of engineering and bioengineering uses. Titanium dioxide nanoparticles are often consumed as a material for orthopedic implants. TiO<sub>2</sub> in powder form is also commonly used as a whitener in toothpastes<sup>(15, 16)</sup>. Titanium dioxide is the most extensively used white pigment because of its brilliance and refractive index for it which is very high, in which it is exceeded just by a few other materials. About 4.6 million tons of pigmentary TiO<sub>2</sub> are used yearly, and this number is likely to increase as application continues to rise<sup>(17)</sup>. There is no study about the effect of TiO<sub>2</sub>NPs on ALP

activity in saliva of gingivitis patients, so we decided to investigate this effect in this research.

## **MATERIALS AND METHODS**

### **Nanoparticles**

Titanium dioxide nanoparticles have been obtained from Hongwunanmter, china. This product supplies as TiO<sub>2</sub> Nano powder. Absorbance spectra of NPs solution were measured by UV-VIS spectrophotometer. All spectra were measured at room-temperature in a quartz cell with 1 cm optical path. Structure and nano size measurement of nanoparticles samples were identified by the Scanning Electron Microscope SEM (Electronic Microscope Centre-Collage of applied Science, University of Technology, Iraq).

### **Salivary Alkaline Phosphatase assay:**

Alkaline phosphatase activity was measured to determine the best saliva volume for this experiment by using different volume of sample (20, 40, 60, 80 and 100)  $\mu$ l. The salivary Alkaline Phosphatase activity was spectrophotometrically determined according to the recommendation of the German Clinical Chemistry Association (using the kit of Human company, Germany). The reaction mixture contained a substrate 50 mmol/L p-Nitrophenylphosphate, and buffer contain 1.25 mol/L Diethanolamine buffer (pH 10.35  $\pm$  0.2) and 0.625mmol/L Magnesium chloride in a total volume of 1.250 ml. In the presence of alkaline phosphatase (100  $\mu$ l of saliva), p-Nitrophenylephosphate is reduced to p-nitrophenol. The rate of decreased in absorbance at 405 nm is directly proportionate to the ALP activity in the sample.

### **Collection of Saliva**

Unstimulated saliva of 25 patients with gingivitis from teaching hospital of College of Dentistry / University of Baghdad were collected. The diagnosis of the patients was done by the following assessment parameters: Gingival index (Loe and Sillness)<sup>(18)</sup>, and Plaque index (Sillness and Loe)<sup>(19)</sup>. Another 20 healthy samples were collected from dental students with average age about (22-23 years) for both groups. Collection was performed 2-3 hours after the volunteer usual breakfast time and after thoroughly rinsing the mouth with water. Saliva was collected by standard spitting method from both groups: control and gingivitis group then saliva collected in a plane tube, centrifuged 10 minute at 1500 xg, and the supernatant liquid was used for chemical analysis.

**Effect of TiO<sub>2</sub> nanoparticles on salivary ALP activity:**

Stock solution of (200 µg/ml) concentration of TiO<sub>2</sub> NPs was prepared and then the following concentrations (10, 20, 30, 50, and 100) µg/ml are prepared by diluting with the same solvent. The enzyme ALP activity was measured in human saliva by using 100 µl of saliva in the same method with replace 20 µl of the solvent (3:1,water:ethanol) with 20µl of TiO<sub>2</sub> NPs solution . The percentage effect on activity was calculated by comparing the activity with and without the TiO<sub>2</sub> NPs and under the same conditions according to the following equations:

$$\% \text{ inhibition} = 100 - 100 \times (\text{Activity in the presence of nanoparticles} / \text{Activity without the nanoparticles})$$

A constant final concentration of TiO<sub>2</sub> NPs (0.15µg/ml) was used to measure the enzyme activity in saliva samples of gingivitis patients and control persons.

**Statistical analysis**

Statistical analysis was performed using SPSS (version 14) and Microsoft Office Excel (Microsoft Office Excel for windows; 2010). Data were analyzed by using Two Way Analysis of Variance (ANOVA). Student T-test was used to assess significant difference among means at level (P < 0.05).

**RESULTS AND DISCUSSION**

Table 1 show the obvious difference between control group and persons with gingivitis in gingival and plaque index.

Figure 1 show the difference in mean and standard deviation for plaque index and gingival index in healthy control person (PLI, GI) and patient with gingivitis (PLI2, GI2). The increase in the two main parameters considers from the main causes of gingivitis and this is proved in many studies.

Figure (2) shows the UV-VIS absorption spectra that indicated the characteristic absorbance feature of titanium dioxide nanoparticles, illustrate absorption spectra of titanium dioxide nanoparticles, the surface related peak could be clearly distinguished. This peak was around 240 nm. This result is agree with that of Chen et al (20), which indicate the absorbance intensity of GalA-TiO<sub>2</sub>NPs dispersion in the UV region in <300 nm.

Figure (3) shows SEM pictures and size distributions of titanium dioxide nanoparticles using in this research. The nanoparticles thus produced were calculated to have the average diameters of 30 nm.

From the results in figure (4), it concluded that 100 µl of saliva is the best volume for measuring ALP activity according to the conditions of experiments.

The kinetic biochemical tests revealed that NPs of TiO<sub>2</sub> caused inhibition effect on salivary ALP activity as shown in figure (5). Observed results show that any increase in TiO<sub>2</sub>NPs concentration caused decreasing in percentage of inhibition of enzyme activity. The greater inhibition of TiO<sub>2</sub>NPs on enzyme activity was at concentration (0.15) µg/ml in total volume 1.370 ml of solvent as shown in figure (6).

In recent study for Al-Rubaee et al.,<sup>(21)</sup> on the effect of Au and Ag nanoparticles on acid phosphatase activity, similar inhibition effect was obtained, the greater inhibition of Au NPs on ACP activity in sera of healthy subjects was 5% at concentration 5.7 µg/ml and Ag nanoparticles was 5.8 % at concentration 10 µg/ml. Another study on the effect of silver nanoparticles on salivary LDH activity observed that any increase in Ag nanoparticles concentration caused increasing in percentage of inhibition of enzyme activity and the greater inhibition of Ag NPs on enzyme activity was 40.1% at concentration 7.5 µg/ml<sup>(22)</sup>.

Schug et al.,<sup>(23)</sup> results show that in intact heterotrophic biofilms, alkaline phosphatase activity was not affected following exposure to surface functionalized TiO<sub>2</sub> NPs and UV radiation. While, an alkaline phosphatase enzyme isolated from E. coli was powerfully inhibited at lower concentrations of titanium dioxide nanoparticles than the intact biofilms.

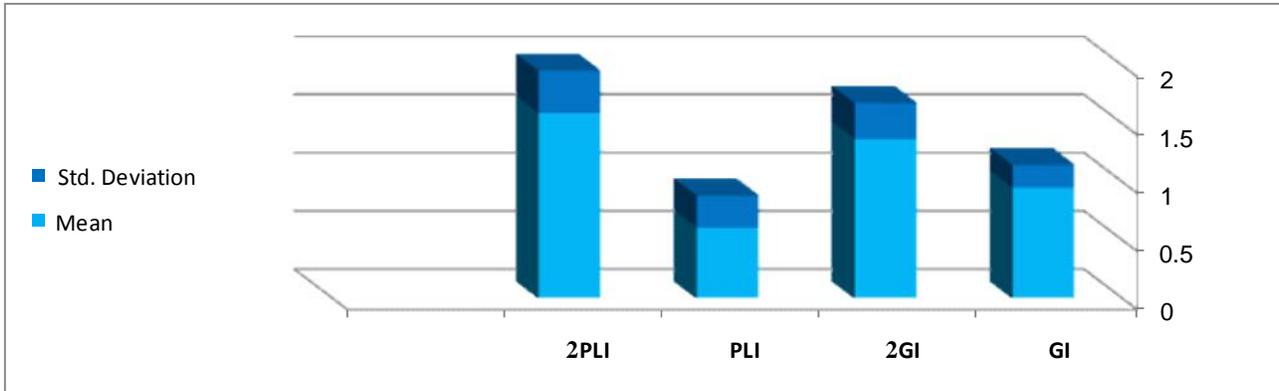
We need many studies in order to investigate and explain the cause of high inhibition of TiO<sub>2</sub>NPs on ALP activity in lower range of concentrations, and determine the type of inhibition.

To try to realize the possible effect of TiO<sub>2</sub>NPs on salivary ALP activity in patients with gingivitis the activity measured with and without NPs and compared with that of control group .The results shown in table(2).

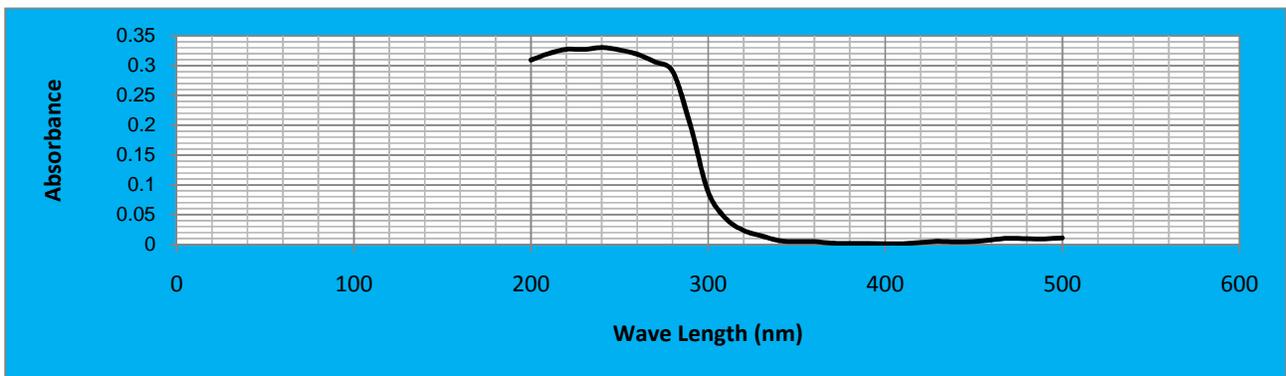
Statistical analysis comparing between the ALP activity of the three groups of this study revealed a significant difference (P 0.05) between control and gingivitis as the ALP activity increase in gingivitis. This result agree with many results from other studies as in Yoshie et al,<sup>(24)</sup> that shown an increase in the alkaline phosphatase activity in the severe stage of

**Table 1:** Summary of GI and PI Scores

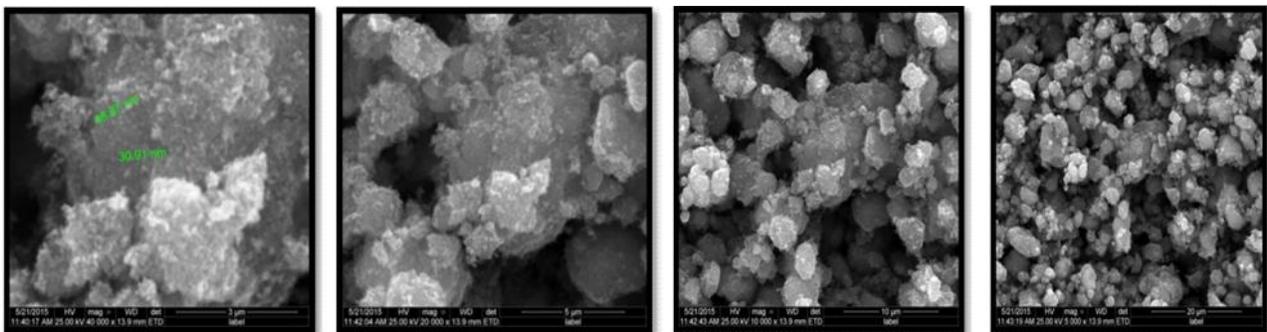
	N	Gingival Index (Mean ± SD)	Plaque Index (Mean ± SD)
Control persons	20	0.95±0.20	0.61±0.27
Patient with Gingivitis	25	1.37 ± 0.31	1.59 ± 0.36



**Figure 1:** The difference between control in plaque index (PLI) and gingival index (GI) and gingivitis group (PLI2, GI2).



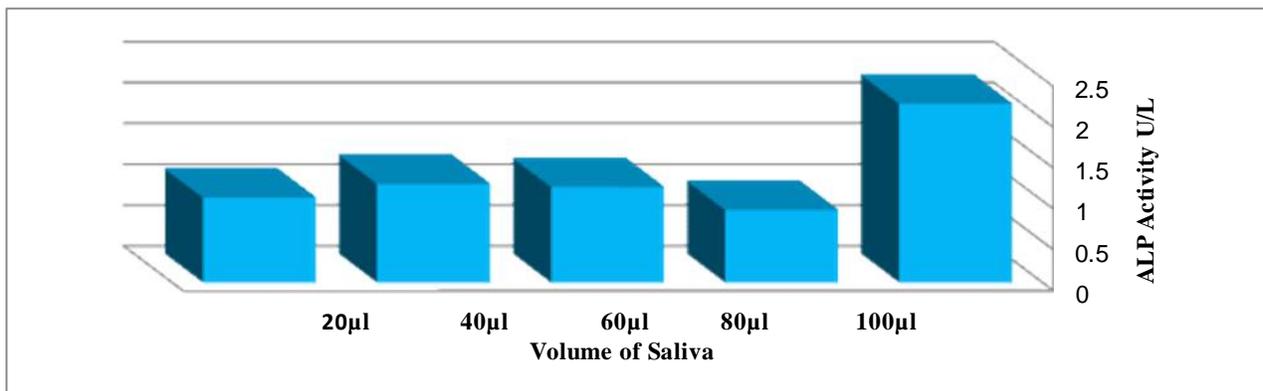
**Figure 2:** Absorbance spectra of the titanium dioxide nanoparticles



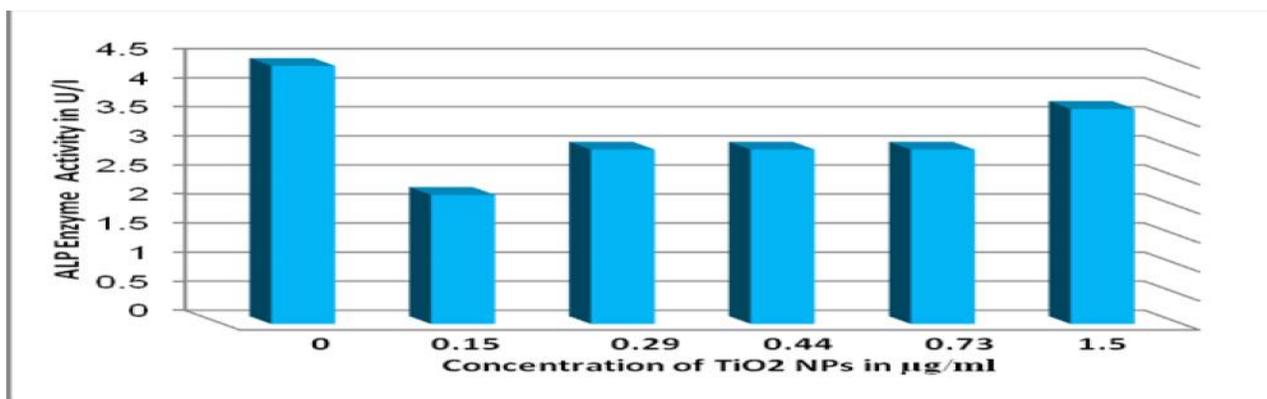
**Figure 3:** shows SEM pictures and size distributions of titanium dioxide nanoparticles.

**Table 2 :** Mean and standard deviation for salivary ALP activity in Gingivitis patients with and without TiO<sub>2</sub>NPs and control group.

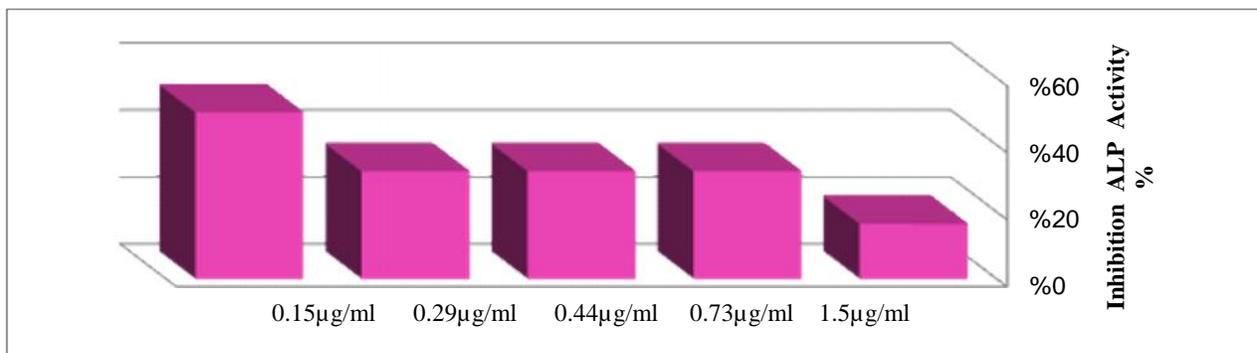
Groups	Salivary ALP Activity in U/L Mean ± SD
Control group	0.74±0.77
Gingivitis group without NPs	1.49±1.34
Gingivitis group with NPs	0.72±0.76



**Figure 4:** Effect of saliva volume on ALP activity



**Figure 5:** Salivary ALP activity in presence of different concentration of TiO<sub>2</sub>NPs.



**Figure 6:** Percentage inhibition of salivary ALP activity in different concentrations of TiO<sub>2</sub>NPs

periodontal diseases, and after treatment, the activity of this enzyme was restored to the normal value found in healthy persons. There are some suggestions explain this increase. Yan et al,<sup>(25)</sup> show the increase in ALP enzyme activity may be due to their release by inflammatory cells and bacterial cells into gingival crevicular fluid and consequently into saliva. This result agree with Numabe et al, <sup>(26)</sup> who show that changes in enzymatic activity of salivary ALP activity reflect metabolic changes in the gingiva in inflammation <sup>(26)</sup>. While other observations <sup>(26, 27)</sup> suggest that a significant amount of alkaline phosphatase levels present in saliva is produced locally by diseased periodontal tissues. ALP levels can be considered as potential indicator for periodontal disease <sup>(27, 28)</sup>. Mojgan and Afsanehin <sup>(29)</sup> show that there is unusual conditions, like loss of alveolar bone due to periodontitis, the activity of salivary ALP may demonstrate a significant rise, which is very important biomarker for periodontal problems.

The same statistical analysis comparing between the (gingivitis - TiO<sub>2</sub>NPs) group, a highly significant difference P 0.001 as a result for inhibition of ALP activity by titanium dioxide nanoparticles. While for the third group (Control - TiO<sub>2</sub>NPs) statistical analysis revealed a nonsignificant difference P 0.05 as the ALP activity increase in gingivitis and inhibited by TiO<sub>2</sub> nanoparticles.

In conclusion, salivary ALP activity was inhibited, a highly significant, by lower concentration of TiO<sub>2</sub>NPs (0.15 µg/ml) more than the upper range of concentrations and this need another study to explain it. Enzyme levels in saliva of gingivitis patients were significantly high than control.

**Table 3:** Significance level among the three groups\*

	df	P value
Control - Gingivitis	19	0.036
Gingivitis - TiO <sub>2</sub> NPs	19	0.000
Control - TiO <sub>2</sub> NPs	10	0.916

\* Control group which represent measurement the salivary ALP activity in healthy subject without TiO<sub>2</sub>NPs and gingivitis group, that represent ALP activity in patients with gingivitis without (TiO<sub>2</sub>NPs) and the third group, salivary ALP activity in patients with gingivitis with (0.15µg/ml) of TiO<sub>2</sub>NPs as a final concentration

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