

Comparison the Activity of ALT Enzyme in Saliva of Periodontitis Patients with Control and Determine the Effect of ZnONPs on its Activity

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Abstract: *The optical and structural properties of the zinc oxide nanoparticles [ZnO NPs] have been studied using SEM and [UV-Vis] spectrophotometer. The produced nanoparticles have molecules size <80 nm and spherical in shape. The zinc oxide NPs effect was investigated on Alanine Transaminase [ALT] activity in the saliva of 20 patients with chronic periodontitis disease as compared with 15 healthy persons with age range from 30-60 years for all groups. The consequences for this study was there is significant increase of salivary alanine transaminase enzyme activity in patients with chronic periodontitis when compared to control subjects, and there is significant elevation in the activity of ALT by the effect of ZnONPs when compare with the same chronic periodontitis patients without using ZnONPs, in conclusion ZnONPs causes activation to ALT enzyme activity.*

Keywords: ZnO nanoparticles, ALT activity, Chronic periodontitis

1. Introduction

Periodontal disease is chronic disease of the mouth consist of a group of inflammatory status affecting the dentition and it's the supporting structures^(1, 2). Relying on the severity of the problem and the appearance period, periodontitis is categorized in three important classes, involving chronic and aggressive periodontitis, in addition to periodontitis related to systemic disease⁽³⁾. Periodontal problems are one of the most common and important disorders of adult's gum. The periodontitis severity could be determined on the basis of its clinical parameters. These parameters consist of depth of periodontal probing pocket depth, loss of clinical attachment and bleeding amount of the mouth. In the last decades, periodontitis had attracted attention because of the use of salivary laboratory tests in the diagnosis of this disease^(4, 5). The saliva might contain ALT (Alanine aminotransferase) as an easily measured test for treating and watching periodontitis reliably. The ALT level in saliva has an important role clinically as a marker of periodontitis as shown by Rai *et al*, because it can reflect destruction and inflammation of periodontal tissue⁽⁶⁾. New scientific tools are nanoparticles which can be used in many pharmacological and biotechnological fields. In recent world nano structural of ZnO has been useful in the different biomedical applications. Because the metal oxide Nanoparticle are stable and with salient properties, they are considered to be safe for applications⁽⁷⁾. Antibacterial activity increases with ZnO nanoparticle⁽⁸⁾. The object of the present study was to determine the activity of salivary ALT enzyme periodontitis patients and compared it with control and determine the effect of ZnO nanoparticles on its activity in saliva of chronic periodontitis patients.

2. Material and Method

1. Nano particle

Zinc oxide nano particle have been received from china, Nanjing. This product obtains as powder of ZnO Nano absorbance spectra of NPs stock solution were measured by UV- VIS spectrophotometer. Nano size measurement of ZnO NPs power and Structure were recognized by Scanning Electron Microscope SEM (Electronic Microscope Center in university of Technology, College of applied Science, Iraq).

2. Salivary Alanine aminotransferase assay

The activity of Alanine aminotransferase was measured to decide the best volume of saliva for this assay by using various volume of sample 20, 40, 60, 80 and 100µl. The activity of Alanine aminotransferase in saliva was determined by using spectrophotometer according to the guidance of Association of German Clinical Chemistry using the kit of Human Company, Germany. The mixture of reaction have a substrate 90 mmol/ L 2- Oxoglutarate, and a buffer contained 150 mmol/L TRIS buffer (pH=7.5) and 750 mmol/L- Alanine. In the presence of alanine aminotransferase [100 µL of saliva], 2- oxoglutarate is reduced to L- glutamate.

3. Collection of Saliva

Saliva used in this study was unstimulated type. It was collected from 20 patients with periodontitis attending teaching hospital of Dentistry College / Baghdad University for treatment. The diagnosis of the periodontitis patients was carried out by following parameters: Gingival index [Loe and Sillness]⁽⁹⁾, and Plaque index[Sillness and Loe]⁽¹⁰⁾. The control group consist of 15 healthy samples were took from dental staff with Average age about 30-60years for both

groups. Collection was accomplished during 2-3 hours after breakfast and ask the volunteer torinse the mouth with water. Saliva was collected by spitting method from both groups: periodontitis and control groups then saliva collected in a test tube, centrifuged 10 min at 4000 rpm, and the supernatant liquid part was used for chemical investigation.

4. Effect of ZnO nanoparticles on salivary ALT activity

Stock solution of [100 µg/ml] concentration of ZnO NPs was prepared and after that the 20 µg/ml are prepared by make dilution with the same solvent. The activity of ALT enzyme was calculated in saliva by using 100 µl of saliva in the like method with replace 20 µl of the solvent [3:1, water: ethanol] with 20 µl of ZnO NPs solution. A constant final concentration of ZnO NPs [0.15 µg/ml] was used to measure the activity of enzyme in samples of saliva of periodontitis patients and control subjects.

5. Statistical analysis

Statistical analysis was accomplished by using Statistical Package for the Social Sciences (SPSS) version 14 and Microsoft Office Excel. Data analysis by using One Way Analysis of Variance [ANOVA]. The Student t-test was used to evaluate significant difference among means at level [P < 0.05].

3. Results

Figure 1 reveals SEM pictures and size distributions of zinc oxide nanoparticles using in this research. The zinc oxide nanoparticles produced were determined to have the average diameters of 80 nm.

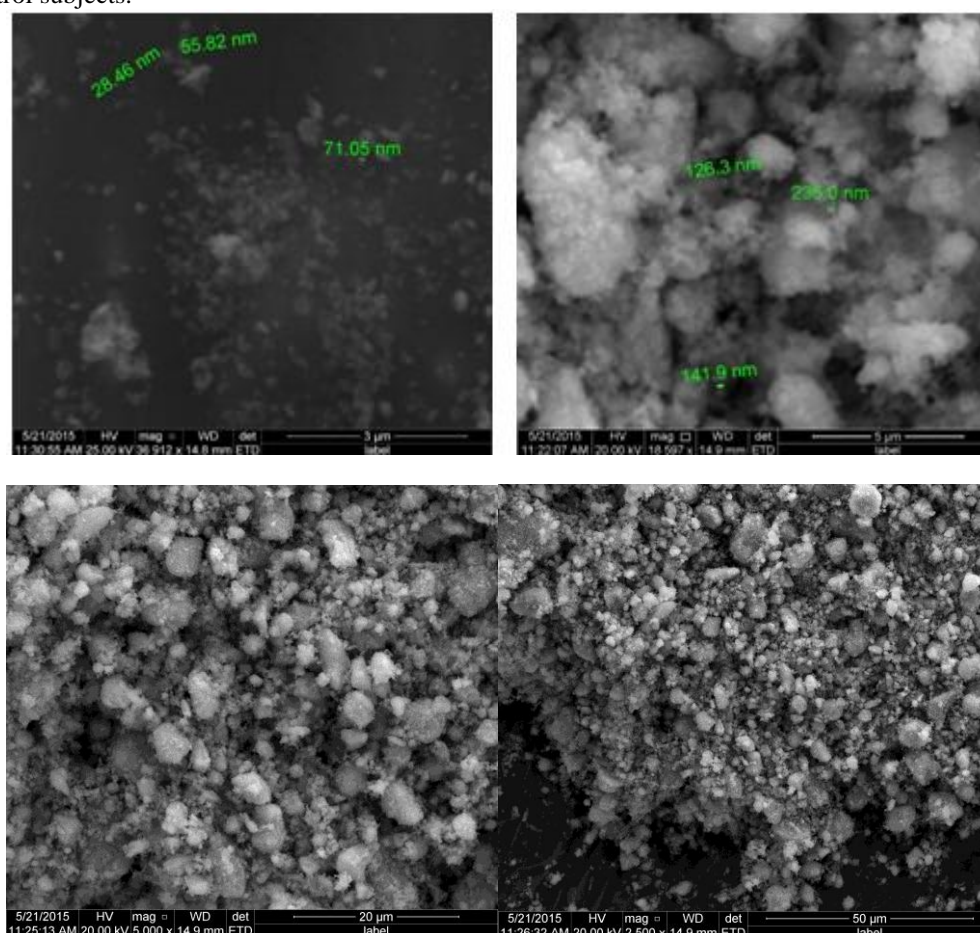


Figure 1: SEM pictures and size distributions of Zinc oxide nanoparticles

From the results in Figure 2, it is found that 100 µl of saliva is the appropriate volume for calculating the activity of salivary ALT according to the conditions of experiments.

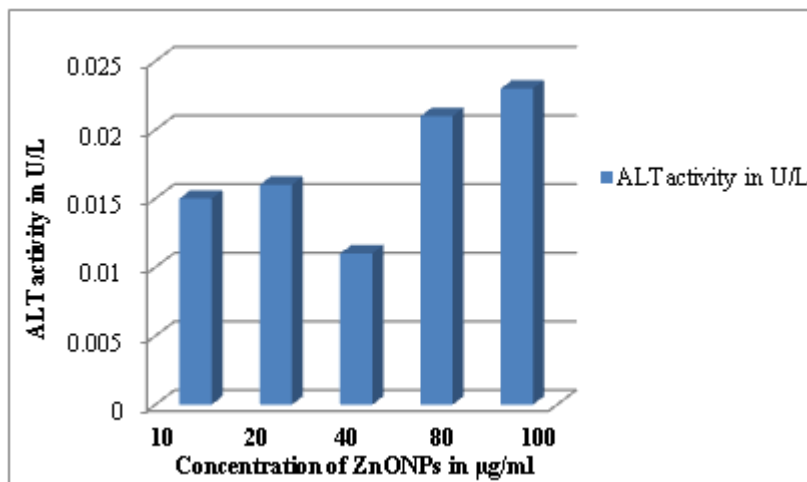


Figure 2: ALT activity in relation to different concentrations of ZnONPs

The results of this study show that the activity of ALT among chronic periodontitis patients without ZnO nano particles was less than its activity among chronic periodontitis patients with nano particles this on one hand and on other hand its activity among control group less than both group: chronic periodontitis with and without nano particles as show in table (1) and figure (3).

Table 1: Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Control	15	1.26	4.22	2.45	1.08
Withoutnano	20	3.11	6.32	4.81	0.99
Withnano	20	4.52	8.31	6.25	1.16

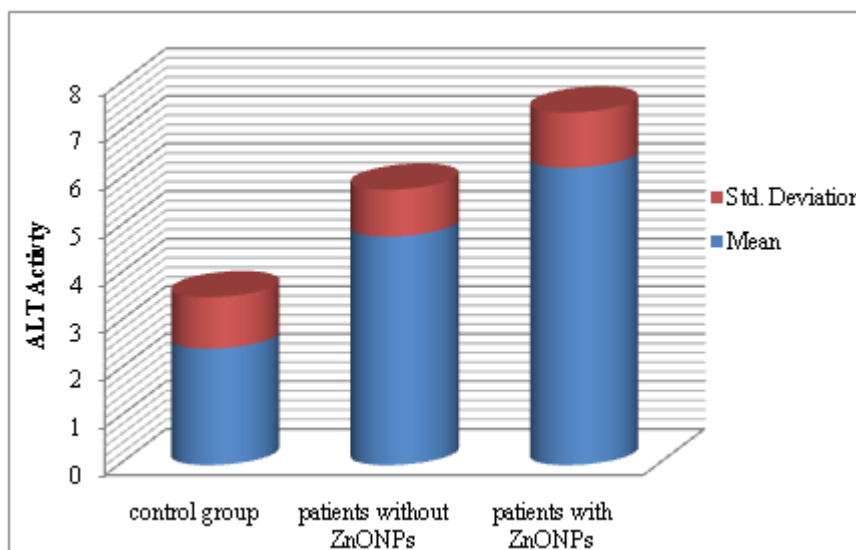


Figure 3: The difference among the three groups in ALT activity

The present study revealed that the multiple comparison by anova test for ALT activity between three groups (control, periodontitis with nano particle, and periodontitis without nano particle) there was highly significant differences. Also there were highly significant differences in ALT activity between two groups: periodontitis without nano particle and periodontitis with ZnONPs, as show in table (2).

Table 2: ANOVA test for ALT activity

	Sum of Squares	d f	Mean Square	F	Sig.
Between Groups	105.78	2	52.89	45.25	0.000
Within Groups	54.94	47	1.169		
Total	160.72	49			

In regard to differences in ALT activity between the control group and both periodontitis without and with nano

particles, current study show that there is highly significant differences.

Table 3: Multiple Comparisons between the three groups

Groups	Mean Difference	P-value	Sig.
Control and patients without nano	2.356	0.000	HS
Control and patients with nano	3.701	0.000	HS
Patients without nano and patients with nano	1.344	0.003	HS

4. Discussion

Quantitation of ALT activity can be easily determine in sample of saliva in recent studies^(11, 12 and 13). The use of saliva to measure the activity of ALT enzyme produces many advantages over GCF (Gingival cravicular fluid). Because the saliva collection needs no specialized

techniques or equipments, it is more convenient and faster for the practitioner and the patients to collect. In addition to that, whole saliva considers a pooled sample from all periodontal sites, and therefore analysis of the biomarkers in the whole saliva may give a complete assessment of the status of disease as compared to analysis of site-specific GCF⁽¹⁴⁾. So this study shows that the activity of ALT in periodontitis groups was higher than control group and this coincides with study conducted by Dabra *et al.* (2012), since the obtained results of Darba study show that the activity of the ALT enzymes in the saliva of the patients with periodontal disease was significantly higher as compared to the control group⁽¹⁵⁾. And they attributed the cause of this result to that this salivary ALT enzyme is considered an intracellular enzyme and included in the cell metabolic processes and it is mostly found in the cells of soft tissues. This enzyme is an indicator of higher levels of damage in the cell and its increased activity in GCF is a result of its increased release from the damaged cells of the soft tissues of the periodontium, and is a reflection of the changes in metabolism of the inflamed gingiva⁽¹⁶⁾. ZnONPs had antibacterial action⁽¹⁷⁾ that cause to choose it to patients with chronic periodontitis inflammation. There are many studies that proved the activation of ALT enzyme under the effect of ZnONPs with simple differences. The result of this study shows that the ZnO nanoparticle increases the enzyme activity and this result resembles the result obtained by Pandurangan and Kim (2012), their present study showed that ZnO nanoparticles increased these enzyme activities⁽¹⁸⁾. Also agree with Fazilati (2013), who showed that ZnONPs (25-200 mg) had increased activity of ALT enzyme significantly at ($P < 0.05$) in serum male rats⁽¹⁹⁾. Sharma *et al.* (2009) has been reported that, level above 50 mg/kg of zinc oxide nanoparticles stimulate the oxidative stress and increase the plasma level of ALT⁽²⁰⁾.

5. Conclusion

The conclusion from this study was that there is significant increase in ALT activity in patients with chronic periodontitis in comparison to control subjects, and also there is significant increase in ALT activity under the effect of ZnONPs as compared with the same chronic periodontitis patients without using ZnONPs, that is, mean ZnONPs causes activation to ALT enzyme activity.

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