



EFFECT OF ZnO NANOPARTICLES ON AST ACTIVITY IN GINGIVAL CERVICULAR FLUID OF CHRONIC PERIODONTITIS PATIENTS: *IN VITRO* STUDY

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ABSTRACT

Medical applications of nanoparticles (NPs) have been studied in different areas to estimate the effect of these nanoparticles on the studied parameters. In this work, the effect of ZnO NPs on Aspartate aminotransferase (AST) activity in Gingival Cervicular Fluid (GCF) of patients with chronic periodontitis was studied. GCF samples of 25 persons, aged (30-60) years with chronic periodontitis were collected as patients group. Powder of ZnO NPs (< 80 nm) was used in this study. The AST enzyme activities in GCF were estimated in patients group without ZnO NPs as a relevant biomarker of chronic periodontitis. The effect of ZnO NPs on AST activity in GCF of patients with chronic periodontitis was evaluated in terms of group with NPs. AST activity was estimated by colorimetric method. Results were showed that AST activity in patients group with ZnO NPs was higher than its activity without ZnO NPs. The effect of ZnO NPs on AST activity in GCF may be attributed to the vital role of ZnO NPs in resistance of the pathogens, in another hand, this effect may be reflects the conformational changes on protein structure after interaction with ZnO NPs.

KEYWORDS: aspartate aminotransferase, Gingival Cervicular Fluid, ZnO NPs and Chronic periodontitis.

INTRODUCTION

Nanoparticles have a greater surface area per weight in compared to the large particles. This property renders the result nanoparticles more active powder^[1]. Recently, effect of nanoparticles of gold, silver and TiO₂ NPs on salivary acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) was evaluated^[2-4]. Recent studies found that nanoparticles of zinc oxide possess biological activity toward some types of pathogenic bacteria^[5-7]. As it was known, the periodontal diseases are bacterial infections of the gingiva and other attachment fibers that support the teeth^[8]. Zinc oxide nanoparticles are found to be nontoxic, biosafe, and biocompatible and have been found benefit as drug carriers, cosmetics, and fillers in medical materials. On the other hand, ZnO nanoparticles that used commercially have advantages, such as lower cost and white appearance^[9]. Zinc oxide powder inter in many products including lubricants, paints, ointments, adhesives, pigments and fire retardants as an additive substance^[10]. Periodontal disease is found by a local accumulation of bacteria and their metabolic products (e.g., endotoxin), that stimulate the junction epithelium to proliferate and produce tissue- destructive proteinases^[11]. Gingival crevicular fluid (GCF) arises at the gingival margin and is otherwise termed transudate or exudate. The flow rate is related to the degree of gingival inflammation, and a rate of 0.05 to 0.20 μL per minute was reported during minimal inflammation. Several studies have been performed on the composition of gingival crevicular fluid and the changes seen during orthodontic tooth movement^[12, 13]. GCF is the fluid between the sulcus or periodontal pocket and the surface

of the tooth and the gingival epithelium. In healthy gingiva, small amounts of this fluid affect the transudate of gingival tissues. During the course of periodontal disease, this fluid is transformed into an inflammatory exudate^[14]. Complex mixture of substances derived from serum, leukocytes, periodontal cells and oral bacteria have been found in GCF. The host-derived substances present in GCF include antibodies, cytokines, enzymes and tissue degradation products^[15]. The volume of GCF has been found to be affected by the status of periodontal disease and is an indicator of gingival inflammation^[16,17]. In medicine the enzyme Aspartate aminotransferase or glutamic oxalotransferase (GOT) is a useful marker for the cell death that occurs in cardiac muscle after a myocardial infarction or in the liver during hepatic disease. After tissue damage, aspartate aminotransferase is released from injured and dead cells into extracellular fluid and can be assayed in serum, tears and in oral cavity in Gingival crevicular fluid and Saliva. The purpose of this study was to evaluate the relationship between Aspartate aminotransferase (AST) levels in gingival crevicular fluid with and without ZnO NPs in periodontal disease .

MATERIALS & METHODS

GCF samples were obtained from 25 patients, aged (30-60) years with chronic periodontitis collected as patients group all of them had no history of any systemic disease, they were well informed about the aim of investigation and they were free to accept or refuse to be examined and they were selected from subjects attending periodontal department in the college of dentistry at Baghdad university.

Periodontal assessments

The periodontal examination were done on a dental chair, the periodontal variables were recorded on four sites (mesial, distal, buccal and lingual) for all teeth and parameters include: plaque index (PI) [18], gingival index (GI) [19], probing pocket depth (PPD):- is defined as the distance from the gingival margin to the most apical penetration of periodontal probe inserted in to the gingival crevice. Clinical attachment loss (CAL): Is defined as the distance from cement enamel junction to the location of the inserted probe tip. Bleeding after probing to the base of the probeable pocket (BOP) has been a common way of assessing presence of sub gingival inflammation [20]. In this dichotomous registration, 1 is scored in cases where bleeding emerges within 15 seconds after probing.

Nanoparticles

Zinc oxide nanoparticles have been obtained from Nanjing, china. This product supplies as ZnO Nano powder absorbance spectra of NPs stock solution were measured by UV- VIS spectrophotometer. Structure and nano size measurement of ZnO NPs powder were identified by the Scanning Electron Microscope SEM (Electronic Microscope Centre- College of applied Science, University of Technology, Iraq).

Collection of GCF

GCF samples were taken from patients in the second visits of periodontal treatment, the patient had received supra gingival scaling and polishing and received good oral hygiene instructions in the first visit to avoid bleeding occurrence during the collection of gingival fluid. Teeth had a pocket depth more or equal to 4mm. prior to the sampling the teeth were thoroughly cleaned from plaque without causing damage to the gingivae. Next, the teeth

and gingivae were carefully dried before the collection of the exudates started. A previously weighed strips of filter paper size 30 were gently inserted in to the selected pocket depth until resistance was felt the filter paper left in place for 30 seconds and after removal they were weighed on a chemical balance, The difference between the weights of filter paper before and after absorption of exudates was calculated and each filter strips was placed in a tube containing 0.3ml of normal saline then transferred and stored at -20C [21].

GCF aspartate aminotransferase assay

Colorimetric method (Reitman and Frankel) was used to determine aspartate aminotransferase activity. The measurement was conducted by monitoring the concentration of oxaloacetate hydrazone formed from oxaloacetate with 2, 4 dinitrophenyl – hydrazine. The activity is determined by using spectrophotometer at absorbance =546nm, and using the kit of Randox Laboratories Limited, Country Antrim.

Effect of ZnO NPs on AST activity in GCF

Aspartate aminotransferase activity in GCF was determined by colorimetric method. Stock solution of (300 µg/ml) concentration of ZnO NPs was prepared. The following concentrations (5, 10, 20, 40, 80, and 100) µg/ml were prepared by diluting with the same solvent. The enzyme activity was determine by using 100µl of GCF and 20µl of ZnO NPs solution, the same steps were conducted for another run without NPs to evaluate the effect of NPs on the enzyme activity by adding 20µl de-ionized water.

The percentage ratio of activation on activity was calculated by comparing the activity with and without the ZnO NPs according to the following equation:

$$\text{activation\%} = 100 \frac{\text{Activity in the presence of nanoparticles}}{\text{Activity without the nanoparticles}} - 100$$

The final concentration of ZnO NPs (0.33µg/ml) was used to identify the enzyme activity in GCF samples of chronic periodontitis patients.

Statistical analysis

Data were analyzed using SPSS software version 19. Descriptive statistics including medians, means, standard deviations, minimum, maximum values and Spearman's rank correlation coefficient test (r) were used in this study. Values of P> 0.05, 0.05 P > 0.01, P 0.01 were considered non- significant (NS), significant (S) and highly significant (HS) respectively.

RESULTS & DISCUSSION

AST activity in chronic periodontitis

Table (1) showed mean, standard deviation, and median of clinical parameters (PLI, GI, BOPO, BOPI, PPD, CAL). Table (2) shows a non-significant correlations between AST activity and clinical periodontal parameters, While the correlations between AST and clinical periodontal parameters (BOP,PPD and CAL) a weak negative non-significant correlations were revealed between AST activity without nano and BOP, PPD and clinical attachment level a weak non-significant correlations of AST .

TABLE 1: Descriptive analysis of Clinical Periodontitis parameters in chronic periodontitis patients

variable	number	median	mean	SD	min	Max
PLI	25	1.3	1.42	0.45	0.95	2.3
GI	25	1.1	1.08	0.13	1.13	2.21
BOPO	25	75.32	74.37	13.3	0	80
BOP1	25	24.68	25.63	13.3	10.3	40
PPD	25	3.76	3.88	.21	3.2.	6.5
CAL	25	3.17	3.43	.23	1.7	6.52

TABLE 2: Correlation between AST and periodontal parameters in C.P.

Periodontal parameter		PLI	GI	BOP	PPD	CAL
Activity of enzyme without nano	Pearson r	-0.20	-0.16	-0.18	-0.026	-0.10
	p-value	0.33	0.44	0.51	0.64	0.49
		NS	NS	NS	NS	NS
Activity of enzyme with nano	Pearson r	-0.19	-0.026	+0.031	+0.12	+0.21
	p-value	0.36	0.13	0.88	0.56	0.31
		NS	NS	NS	NS	NS

*Significant NS at (P>0.05) level of significance

Popovic *et al.* [22] agreement with this study, who found that non –significant correlation between AST activity and clinical parameter. In another study [23], it was reported that there was non significant correlation between AST activity and clinical periodontal parameters. Wahab and Ahmed [24] found that there was highly significant strong positive correlation between salivary AST activity and CAL in CP patients. The intracellular of AST activity included in the metabolic processes of cells and they were indicators of a higher level of cellular distraction and a reflection of metabolic change in the inflamed gingival tissue [25-27]. The results obtained in the present study were in agreement with many other studies [28,29]. Similarly, the levels of Aspartate aminotransferase enzyme in gingival crevicular fluid was increased in periodontitis patients in compare healthy and gingivitis patients as it reported

elsewhere [30,31]; Luke *et al.* [32] were found that highly significant difference of AST activity in saliva between CP patients and control groups. In Another hand, Totan *et al.* [30] was estimated the level of AST in saliva from CP patients and, they found that there was highly significant strong positive correlation between salivary AST activity and CAL in CP patients.

UV- VIS absorption spectra

Spectra of UV-VIS were indicated the characteristic absorbance feature of Zinc oxide nanoparticles , the maximum absorption peak of ZnO NPs , which suspended in ethanol-water mixture, was showed at 375 nm as shown in figure (1). This absorption peak considers as a hallmark of ZnO NPs at applied nanoparticles size (<80 nm).

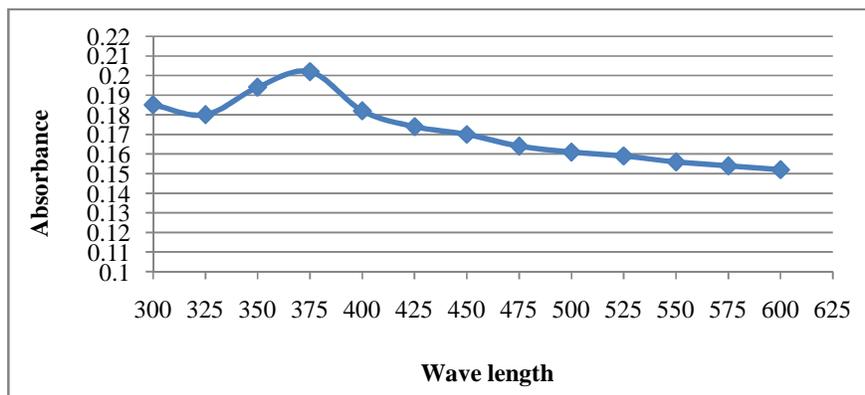


FIGURE 1: UV – VIS spectra of the ZnO NPs

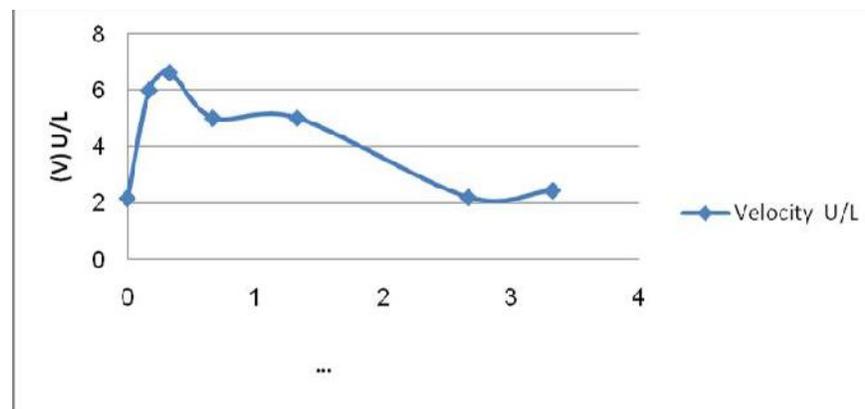


FIGURE 2: AST activity of GCF in presence of different concentration of ZnO NPs

Effect of ZnO NPs on AST Activity in GCF of chronic periodontitis

The results in Figure (2) demonstrated the activator effect of ZnO NPs of AST activity in GCF, figure(3) showed

The greater activation percentage of AST activity by ZnO NPs was found to be of 65.25 at concentration of 0.33 µg/ml, as a more effective NPs concentration among others.

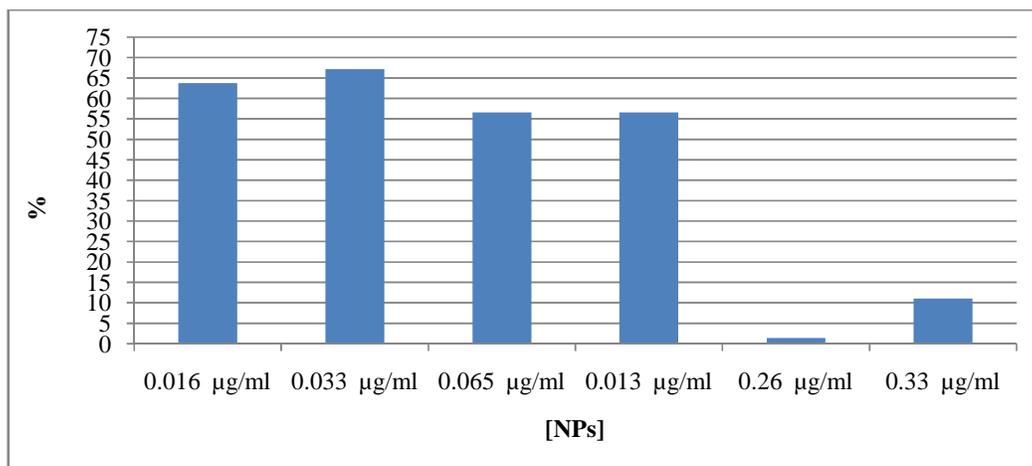


FIGURE 3: percentage activation of AST activity in different concentrations of ZnO NPs.

The AST activity was measured in unit/liter for the studied groups on GCF without ZnO NPs and with ZnO NPs groups. The results of this effect were shown in table (3). AST activity (mean ± SD) in presence of nanoparticles of zinc oxide (3.98 ± 1.40) was higher than its activity in

patient's GCF without NPs (1.125 ± 0.59). Highly significant difference was found to be among the studied groups (p=0.000). These results were illustrated in fig. (4), which was showed the effect of ZnO NPs on AST activity clearly.

TABLE 3: The mean and standard deviation for AST activity in GCF of chronic periodontitis patients with and without ZnO NPs

Groups	No	median	mean	SD	Min	Max	P-value
Activity of enzyme with nano	25	3.14	3.98	1.40	0.98	5.11	0.00 HS
Activity of enzyme without nano	25	1.01	1.125	0.59	0.016	2.1	0.00

* Significant HS at (P 0.01) level of significance

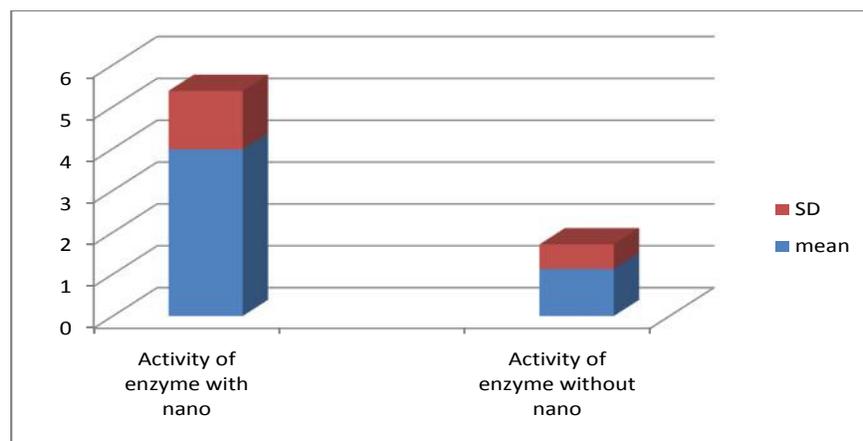


FIGURE 4: Mean and SD of AST activity with and without ZnO NPs in GCF of CP

The present work considers the first study that demonstrates the effects of ZnO NPs on AST activity in GCF of chronic periodontitis patients. Our results indicated that there is activation effect of these NPs on enzyme activity. Pandurangan and Kim showed that ALT, AST, ALP and LDH enzymes activities were significantly increased in a dose-dependent manner by ZnO NPs and significantly produced cytotoxicity in C2C12 cells^[33]. Moderate concentration of zinc oxide nanoparticles leads to an increase in serum LDH activity comparing with

control group in male mice^[34]. In previous study, the activation effects of gold and silver nanoparticles on choline esterase and monoamine oxidase activities were demonstrated^[35]. AL-Rubae^[4] showed that the effect of gold nanoparticles on salivary LDH activity increased with different concentration of the nanoparticles. The effects of gold and silver nanoparticles were studied on the activities of serum AST and ALT enzymes, inhibitor effects were demonstrated, and these effects increased with the increasing of nanoparticles concentrations^[36]. ZnO NPs

have been widely used in production of food and in medicine^[37].

CONCLUSION

It can be concluded from the obtained results of this study that ZnO NPs increased the activity of AST in GCF of CP. This effect may be attributed to conformational changes on protein structure after interaction with ZnO NPs. Several other studies will be needed to explain and understand this effect.

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