Effect of ZnO Nanoparticles on AST Activity in Gingival Cervical Fluid of smokers and Nonsmokers Chronic Periodontitis Patients: in Vitro Study

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Abstract

Objectives: current studies to estimate the effects of cigarette smoking on oral periodontal health, and due to the lack of studies that simultaneously compared the periodontal condition smokers with periodontal disorders, and nonsmokers with periodontal disorders, we assessed the periodontal condition in these two groups. The effect of ZnO NPs on aspartate aminotransferase (AST) activity in Gingival Cervical Fluid (GCF) of smokers and nonsmokers patients with chronic periodontitis was studied as a part of assessment of tissue damage that has been occur due periodontal disease and smoking. Nanoparticles (NPs) have been studied in different areas to evaluate the effect of nanoparticles on the activity of enzyme as a part of more accurate diagnosis. Materials and Methods: GCF samples for 26 persons (aged 35-50 years) with chronic periodontitis were collected as patients group. Powder of ZnO NPs (< 80 nm) was used in this study. The activities of AST enzyme in GCF were estimated in (smoker and nonsmoker) patients group with and without ZnO NPs as a pertinent biomarker of chronic periodontitis. The impact of ZnO NPs on AST activity in GCF of patients with chronic periodontitis was evaluated in terms of group with AST activity was assessed by colorimetric method. Results: results were showed that AST activity in smoker patients group with and without ZnO NPs was higher than its activity in nonsmoker patients. Conclusion: The impact of ZnO NPs on AST efficiency in GCF may be due to the vital role of ZnO NPs in durability against pathogens, in another hand, this impact may be reflects the changes on protein structure after interaction with ZnO NPs.

Keywords: Gingival crevicular Fluid, aspartate aminotransferase , ZnO NPs , smoker and nonsmoker Chronic periodontitis .

Introduction

Nanoparticles have a greater surface area per weight in comparison to the large particles. This property makes the resulted nanoparticles more active powder [1]. Zinc oxide nanoparticles are nontoxic, biosafety, non-harmful to the living tissues and used as drug carriers, and fillers in medical materials [2]. Recently, it was found that nanoparticles of zinc oxide hold biological activity across some sorts of pathogenic bacteria [3, 4]. From the other hand, most ZnO NPs that have been used commercially possess some advantages, in comparison to silver nanoparticle, such as cheaper and white appearance [2]. Periodontal disease is caused by a local accumulation of some pathogenic bacteria in dental plaque and their toxic metabolic products (e.g., endotoxin), that effect the functional epithelium and stimulate its proliferation and production of tissue-destructive proteinases [5]. The severity of periodontitis could be determined on the basis of its clinical parameters such as depth of periodontal probing pocket depth, loss of clinical attachment and bleeding amount of the mouth [6]. During studies it was found that the levels of AST enzyme were significantly higher in saliva of chronic periodontitis patients due to the tissue destruction taking place in these conditions, and this give the importance of using AST activity in saliva for diagnostic purpose [7]. For the last decades, it was believed that smoking is the prime risk factor for the development of periodontal disease, and has its effect on the prevalence, extent, and severity of disease. In addition, smoking can negatively affect the clinical outcome of surgical and non surgical treatment [8, 9]. It has been shown that, after setting of
potential confounding factors, such as, age, oral hygiene, gender, and socioeconomic status, smoking is a main risk factor for periodontal diseases [10]. Gingival crevicular fluid (GCF) termed transudate or exudate arises from gingival sulcus. The flow rate is directly related to the degree of gingival inflammation, and a rate of flow below 0.20 µL per minute was reported during minimal inflammation. Several studies have been completed on the composition of gingival crevicular fluid [11, 12]. GCF composed of a complex mixture of substances acquired from serum, neutrophils, cells of periodontium and oral bacteria. The substances that are present in GCF and host-derived include antibodies, cytokines, enzymes and tissue degradation products [13]. The volume of GCF has been shown to be directly associated with the condition of periodontal disease and can be used as an indicator of gingival inflammation [14,15]. The enzyme Aspartate aminotransferase, also called glutamic oxalotransferase (GOT), in medicine, is powerful marker for the cell death that occurs in cardiac muscle after an attack of myocardial infarction or in the liver during hepatic disease. After tissue damage, the enzyme aspartate aminotransferase is released from damaged and dead cells into extracellular body fluid and can be readily evaluated in serum, tears and in oral cavity (Gingival crevicular fluid and Saliva)[16]. The purpose of this study was to evaluate the relationship between Aspartate aminotransferase (AST) levels in gingival crevicular fluid in smoker and non smoker chronic periodontitis patients ,as indicator of tissues damage, then studying the effect of ZnO NPs on AST activity in GCF of smokers and nonsmokers patients with chronic periodontitis . Nanoparticles (NPs) have been studied in different areas to evaluate the effect of nanoparticles on the activity of enzyme as a part of more accurate diagnosis.

Materials and Methods
GCF samples were taken from 26 patients; they were well informed about the aim of investigation and they were free to accept or refuse to be examined all of them were selected from subjects attending periodontal department in the college of dentistry, University of Baghdad. Their age was (35-50) years with chronic periodontitis. They had no history of any systemic disease, and were divided in to two groups (smokers and nonsmokers) each group composed of 13 patients.

Periodontal Assessments
The periodontal examination were performed in a hospital on a dental chair,and the examination were done by periodontist , the periodontal variables were recorded for the all examined teeth on four sites (mesial, distal, buccal and lingual) these parameters include: plaque index (PI) , gingival index (GI) [17], 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 probable pocket (BOP) has been a common way of assessing presence of sub gingival inflammation [18]. 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.

Collection of GCF
To avoid gingival bleeding at the time of GCF collection the sampling of GCF from chronic periodontitis patients were done in the second visit after patients receiving a good oral hygiene instruction in addition to scaling and polishing in his first visit to the hospital. The GCF were collected from teeth had a pocket depth of 4mm or more, they were cleaned carefully from plaque before sampling without causing any damage to the gingiva to avoid bleeding. The gingiva and teeth were dried very well to avoid contamination of GCF with saliva at the time of sampling, then strips of filter paper size 30 which were weighed previously ,were gently inserted in the selected pocket depth until little resistant was felt ,then after 30 seconds they were removed and weighed again on chemical balance. The weight of fluid were calculated from the difference in weight of filter papers before and after absorption of exudate, then each filter strips was placed in
a tube containing 0.3ml of normal saline then transferred and stored at -20C [19].

GCF Aspartate Aminotransferase Assay

Colorimetric method (Reitman and Frankel) was used to evaluate aspartate aminotransferase activity. The measurement was proceeded by monitoring the concentration of oxaloacetate hydrazone formed from oxaloacetate with 2, 4 dinitrophenyl – hydrazine [20].The activity is assessed by using spectrophotometer at absorbance λ=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 assessed 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.

Result and Discussion

The descriptive statistics for periodontal parameters were shown in table 1, the mean, standard deviation of clinical parameters (PI, GI, PPD, CAL) for smoker and nonsmoker group were shown in the table , the mean of plaque index were higher in smoker (1.54) than nonsmoker group (1.11) while the gingival index was higher in nonsmoker it was (1.15) than smokers (1.05). Also there was increase in the mean of PPD and CAL in smoker than nonsmoker group. T- Test between smoker and nonsmoker group were shown in table 2 there was a non-significant difference between clinical periodontal parameters (PI, GI, PPD) and between AST activity with and without Nano particles .while there was significant difference with the clinical attachment level between the two groups.

Table 1: Descriptive statistics for periodontal parameter of smoker and nonsmoker in periodontitis patients

<table>
<thead>
<tr>
<th></th>
<th>Non smoker</th>
<th>Smoker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>1.11</td>
<td>0.33</td>
</tr>
<tr>
<td>GI</td>
<td>1.15</td>
<td>0.16</td>
</tr>
<tr>
<td>PPD</td>
<td>4.24</td>
<td>1.28</td>
</tr>
<tr>
<td>CAL</td>
<td>4.52</td>
<td>1.41</td>
</tr>
<tr>
<td>min</td>
<td>0.70</td>
<td>0.73</td>
</tr>
<tr>
<td>max</td>
<td>2.4</td>
<td>6</td>
</tr>
<tr>
<td>min</td>
<td>1.00</td>
<td>3</td>
</tr>
<tr>
<td>max</td>
<td>1.5</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2: T- test for clinical parameter and AST activity with and without nanoparticles between smoker and nonsmoker groups

<table>
<thead>
<tr>
<th></th>
<th>PI</th>
<th>GI</th>
<th>PPD</th>
<th>CAL</th>
<th>AST without nano</th>
<th>AST with nano</th>
</tr>
</thead>
<tbody>
<tr>
<td>t test</td>
<td>0.116</td>
<td>0.921</td>
<td>0.60</td>
<td>0.008</td>
<td>0.045</td>
<td>0.036</td>
</tr>
<tr>
<td>p-value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

In Table (3) t test and descriptive statistics were done for AST enzyme activity in both groups with and without nanoparticles and there was significant differences between two groups as shown in the table at p values <0.05.
In Table 4 there was a non-significant correlations between AST activity and all clinical periodontal parameters .The correlations between AST with and without nano of both groups and clinical periodontal parameters (GI, PPD and CAL) was weak negative non-significant correlations. While GI and PI positive non-significant correlations of AST with nano of smoker and nonsmoker patients.

Intracellular enzymes such as AST, is highly released from the damaged cells of periodontal tissue cells into the GC Fand saliva [21]. Also AST enzymes can assess to evaluate the progression of periodontal disease and it appear to have a great benefit to test the activity of periodontal disease[22] Higher levels of AST were found in the GCF in diseased site, than healthy site. Also significant AST level have been found in gingival and periodontal ligament fibroblast[23]. From the results of this study, no significant difference for AST enzyme level in GCF between smokers and nonsmokers with chronic periodontitis these result agree with Mohammed( 2011) how found non-significant difference in AST level between smokers and nonsmokers in saliva [24].Also these results disagree with Vander et al and Ray et al[25,26]. Also this study disagree with Lekaa et al and others how found high significant difference for salivary ALP, CK, and LDH in smoker than nonsmoker [27,30]. This could be due to the small number of sample selected in the study and different methodology. There was a negative non-significant correlation between AST and clinical periodontal parameters, in both groups. These findings disagree with Herasaki et al [29] how found significant correlation between enzymes and periodontal parameters in saliva. In Another study, they found that there was highly significant strong positive correlation between salivary AST activity and CAL in chronic periodontitis patients[31]. The present study showed a weak negative correlation between CAL and AST levels in GCF reflecting the biological activity that take place in the periodontium during acute and chronic inflammatory response. In the present study statistical analysis of enzyme activity with and without nanoparticles was significant in both smoker and nonsmoker group indicated that nanoparticles can be used as a good modality of measuring enzyme activity. This work considered the first study that demonstrates the effects of ZnO NPs on AST activity in GCF of chronic periodontitis patients in smoker and nonsmoker. Our results indicated that there is activation effect of these NPs on enzyme activity. The AST activity was measured in unit/liter for the studied groups on GCF without ZnO NPs and with ZnO NPs groups. AST activity in presence of nanoparticles of zinc oxide was higher than its activity in patient’s GCF without NPs in smoker and nonsmoker and this was in agreement with Pandurangan and Kim how showed that ALT, AST, ALP and LDH enzymes activities were significantly increased in C2C12 in a dose-dependent manner by ZnO NPs and significantly produced cytotoxicity inC2C12 cells [32]. AL-Rubae[33] showed that the effect of gold nanoparticles on salivary LDH
activity increased with different concentration of the nanoparticles. Kadhim et al.[34] showed that the activity of salivary AST with ZnO NPs was higher than without ZnO NPs in chronic periodontitis patients.

Conclusion

It can be concluded from the obtained results of this study that ZnO NPs increased the activity of AST in GCF in smoker and nonsmoker chronic periodontitis patients. 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.

References


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