Investigation the levels of endotoxin and 8-OHdG in sera of patients with *H.pylori*positive peptic ulcer

Nihayet Bayraktar[1], İslim Güler[1], Mehmet Bayraktar[2], Ismail Koyuncu[1]

[1]Harran University, Faculty of Medicine, Dept. of Medical Biochemistry, Şanlıurfa
[2]Harran University, Faculty of Medicine, Dept. of Medical Microbiology, Şanlıurfa

Abstract

Background and aim:

Peptic ulcer is considered an important public health problem and generally associated with complicated conditions such as bleeding and perforation. The aim of this study is to reflect the rate of oxidative damage in the body among patients with H. pylori positive peptic ulcer by measuring serum 8-hydroxy-2p-deoxyguanosine (8-OHdG) and its association with the level of bacterial endotoxin.

Methods

Patients applied to Harran University Gastroenterology Outpatient Clinic with dyspeptic complaints were enrolled in this study. According to gastrointestinal endoscopy findings, 43 patients with H.pylori positive peptiv ulcer patients and 43 healthy volunteers were included in this study. H.pylori diagnosis was detemined by H.pylori urea breath and stool antigen tests. Serum 8-OHdG and endotoxins were measued by ELISA.

Results

A total of 43 patients with peptic ulcer (13 females 30 males), 43 healthy individuals (16 females 27 males) ages (18- 70) years in the study. In biopsies taken endoscopically; Hp severity was mild in 19 patients (43.9%), moderate in 21 patients (48.5%), and severe in 3 patients (7.6%). 8-OHdG which has the potential to mark DNA damage level in serum samples of patients with H.pylori positive peptic ulcer, was compared with the healthy and patient group. It was observed that there was a statistically significant difference ($p < 0.01$). In addition, a weak correlation was found between OHdG and endotoxin.

sConclusion:

Reactive oxygen species (ROS) produced due to increased endotoxin as a result of H. pylıori infection can attack nucleic acid in infected cells resulting in an increased in serum 8-OHdG level. H.pylori and its endotoxin had a significant in peptic ulcer pathogensis.
Keywords: 8-OHdG, Endotoxin, DNA Damage, *H.pylori*

What is already known about this topic?

H. pylori infects the lower part of the gastric mucosas and it is an effective bacterium in the formation of clinical manifestations. Continous and prolonged infection can result in gastritis, peptic ulcer.

What does this article add?

Endotoxin level increases in patients with H. pylori. It has been determined that increasing endotoxin causes DNA damage by increasing the amount of free radicals. H.pylori and its endotoxin had a significant in peptic ulcer pathogensis.

1. INTRODUCTION

Helicobacter pylori is a Gram negative microaerophilic bacterium that colonizesvarious areas of the stomach and duodenum. As with other gram negative bacteria, it has endotoxins in addition to other virulance factors such as urease enzyme, vacuolating cytotoxin A (Vac A), cytotoxin associated gene (Cag A)[1,2]. The latter triggers interleukin 8 (Il-8) chemochine attracts neutrophils.Thses cells produce protease and reactive oxygen species (ROS)[3]. Despite the acidity of gastric juice, *H. pylori* infects the lower part of the gastric mucosas and it is an effective bacterium in the formation of clinical manifestations[1,2]. Continous and prolonged infection can result in gastritis, peptic ulcer and gastric cancer[2]. H. pylori has also a role in the formation and relationship with gastric MALT (Mucosa Associated Lymphoid Tissue) lymphoma[4]. Thus, its pathogenic importance is increased. It is a matter of concern that the damage in the gastric cellular dimension may be via DNA damage caused by *H.pylori* endotoxins and oher virulence factorsand this explainsthe pathogenicity.Small amounts of

endotoxin can result in fever, inflammation, sepsis, tissue damage[5,6]. ROS induced endotoxins involved in many bacterial diseases including H. pylori infection[7]. ROS easily oxidize guanine bases in DNA and form 8-OHdG. The latter is short term DNA lesion and it is generally regarded as a biomarker of oxidative DNA damage[8-11].

Oxidative stress due to overproduction of ROS can attack cellular macromoleculesand convert them into oxidized forms resulting in DNA damage, protein modification, lipid peroxidation7,12.High Concentration of DNA damages may ultimately lead to activate oncogenes or inactivate tumor suppressor genes such as p53 and Paten. Disturbing of suppressor genes takes place through mechanism of phosphorylation and oxidation13.The sequalae is development of various extra gastrointestinal diseases such as cardiovascular, diabetes mellitus, stroke, chronic inflammation and cancers14-18.

8-OHdG is widely tested in many studies as a biomarker for measuring oxidative DNA damage and as a risk factor for many diseases, including various inflammatory-based gastrointestinal diseases such as gastritis, colitis and esophagitis, inflammatory bowel disease, as well as gastric cancers5-7,10,14,19. The purpose of this study was to determine serum 8-OHdG and endotoxin levels in patients with H. pylori positive peptic ulcer.

2. METARIAL AND METODS

H. pylori positive positive peptic ulcer 43 patients and 43 healthy volunteers were selected from outpatients with gastrointestinal complaints admitted to. Harran University Medical School Gastroenterology Outpatient Clinic for Research. According to the gender and age of the participants; in total, 29 (33.3%) were female and 57 (66.7%) were male, in the patient group, 13 (30.3%) women, 30(69.7%) male; in the control group, 16 (36.4%) were female and 27 (63.6%) were male.The mean age of the total was 31.8% between 18-30 years, 43.9% between 31-49 years, and 24.2% between 50-60 years.The mean age was 40.5 14.3 ± 39.7 13.1 in the control group and 41.3 ± 15.6 in the patient group.Patients with gastroduodenal complaints were undergone gastroscopic examination, urea breath test. Histopathology and stool investigations for H.pylori were done. H.pylori positive peptic ulcer were reported on basis of at least two mentioned tests.

Blood was collected from studied individuals and transferred into biochemical tubes. Blood samples were centrifuged at 3,000 rpm for 10 min, and sera were separated and aliquots of the samples were immediately stored at −80°C until time of the assays.

2.1. Measurement of 8-OHdG in Serum

Quantification of serum 8-hydroxy-2′-deoxyguanosine (8-OHdG) was done by using a commercially available enzyme-linked immunosorbent assay ELISA (Xinfan Biotechnology Co., Ltd, Shanghai, China). All procedures used in the present study followed the instructions strictly. The optical density value was measured with a microplate reader at a wavelength of 450 nm. Then, we calculated the concentration of 8-OHdG and CAT according to the standard curve. All standards and samples were tested in duplicate wells. The inter-assay and intra-assay coefficients of variation for 8-OHdG were 11% and 9%, respectively. The mean minimum detectable dose of human 8-OHdG was 10 ng/L, and the line range was 10–300 ng/ L. The inter-assay and intra-assay coefficients of variation for human CAT were 11% and 9%, respectively, and the mean minimum detectable dose was 3 U/L. The measurement range of the human CAT kit was 3–90 U/L.

2.2. Measurement of 8-OHdG in Serum

In the study, the level of DNA damage is determined by measuring seum 8-OHdG using Enzyme − Linked Immunosorbent Assay (ELISA) (Fine)Test; Wuhan Fine Biotech Co., Ltd., Catalog No. EU2548) for kit. 8-OHdG ELISA Kit) is a competitive assay that can be used for the quantification of 8-OHdG in urine, cell culture, plasma, and other sample matrices. ELISA utilizes an 8-OHdG coated plate and an HRP-conjugated antibody for the detection range of the ELISA test was 1.563 ng / ml to 100 ng / ml, and the detection range was low concentration was below 0.938 ng / ml, with a sensitivity of 0.59 ng/mL. The other highlights of this kit are a quick incubation time of 60 minutes, stable reagents, and an easy kit protocol application.

2.3. Measurement of Endotoxin in Serum

Endotoxin analysis is measured using HEK-BlueTM LPS Detection Kit 2 (InvivoGen; Colorimetric cell-based assay; Catalog: replps 2; Version: 15C04-MT) kits kit protocols applied. Kit protocol was applied according to the manufacturer's recommendations. Designed with endotoxin sensor cells and highly sensitive to LPS HEK-Blue ™ -4 cells are designed HEK293 cells, then express many genes. In addition, NF-κB-inducible, secreted embryonic alkaline phosphatase (SEAP) reporter gene. LPS amount per minute, It is detected by HEK-Blue ™ -4 cells that cause NF-κB activation. These cells are TLR4 via TLR4 pathway. Detection plate, microplate reader at 620-655nm optical density value was determined. Endotoxin concentration of samples was calculated.

Obtained data were transferred to SPSS (Statistical Package for Social Science) programme for statistical analysis. The amount of 8-OHdG in serum samples for DNA damage level and in endotoxin analysis. It was calculated whether the amount of endotoxin was different between the patient and control group.

2.4. Statistical analysis

Data obtained from study samples were presented as means and standard deviation of means (SD) and analyzed by one-way analysis of variance (ANOVA). TUKEY post hoc test on the SPSS (16 package) program was performed. Parametric variables were compared with student t test and Pearsons' correlation coefficient was used for correlation analysis.

Differences among the average values according to $p < 0.01$ were evaluated to be statistically significant.

3. RESULTS

A total of 43 patients with peptic ulcers (13 females 30 males), 43 healthy individuals (16 females 27 males) in the study were ages (18- 70) years. In biopsies taken endoscopically

In patients with peptic ulcer (gastric ulcer + duodenal ulcer) detected in gastroscopy and histopathologically H.pylori positive, biopsy antigen test was H.pylori positive in 17 patients (40.0%) and H.pylori negative in 26 patients (60.0%) of all patients.

Urea breath test results were positive in 40 (93.0%) patients and negative in 3 (7.0%) patients.

8-Hydroxy deoxy guanosine (8-OHdG), a marker of DNA damage level in serum samples of H.pylori positive peptic ulcer patients. Comparison of serum 8-OHdG H. pylori positive peptic ulcer patients and control group (Figure 1).

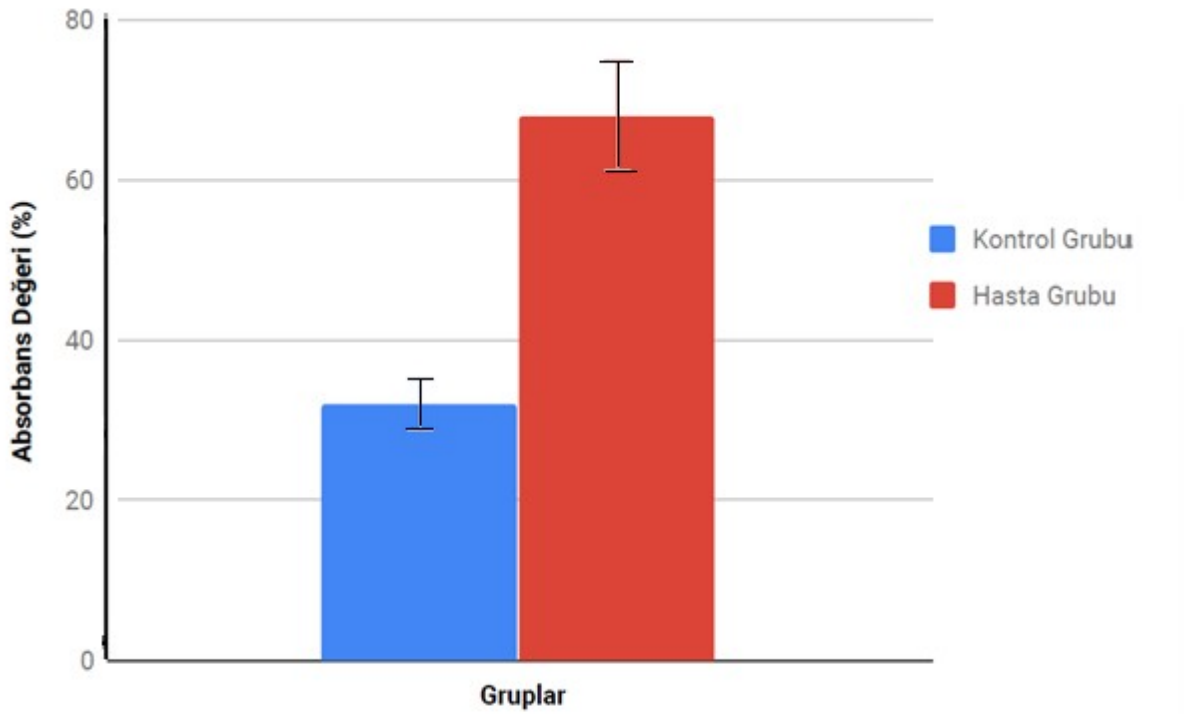

Figure 1: Comparison of serum 8-OHdG levels of H. pylori positive peptic ulcer patients and control groups

In order to determine the level of DNA damage, a statistically significant difference was found when the serum 8-OHdG level was compared between H. pylori positive peptic ulcer patients and control groups ($p < 0.01$; Table 1).

Table -1: The means ± standard deviations of serum levels of 8-OHdGin H.pylori positive peptic ulcer patientsand control groups

Parameters	Patient group(n=43)	Control group(n=43)	P value
8-OHdG (ng/ml)	5.078 ± 1.89	2,37 ± 1.18	p<0.01

Correlation analysis was performed in order to determine whether there is any relationship between these two parameters by evaluating the serum samples of the patients with H.pylori (+) positive peptic ulcer and the control group serum samples after 8-OHdG measurements and endotoxin analysis. Accordingly, a negative weak correlation was observed between 8 - OHdG with DNA damage marker potential and endotoxin levels.

The amount of endotoxin is determined by HEK-Blue ™ -4 cells. HEK-Blue ™ -4 cells are endotoxin sensor cells and highly sensitive to LPS, biologically active endotoxin with the HEK-Blue ™ LPS Detection Kit. These cells express TLR4 and many genes by the TLR4 pathway. In addition, NF-κB-inducible, secreted embryonic alkaline phosphatase (SEAP) reporter gene. HEK-Blue ™ -4 cells with a sensitivity of 0.01 EU / ml and the amount of LPS per minute, which also leads to NF-κB activation. Measured at absorbance at 620-655nm and endotoxin concentration It was calculated.

Comparison of serum endotoxin H. pylori positive peptic ulcer patients and control group (Figure 1).

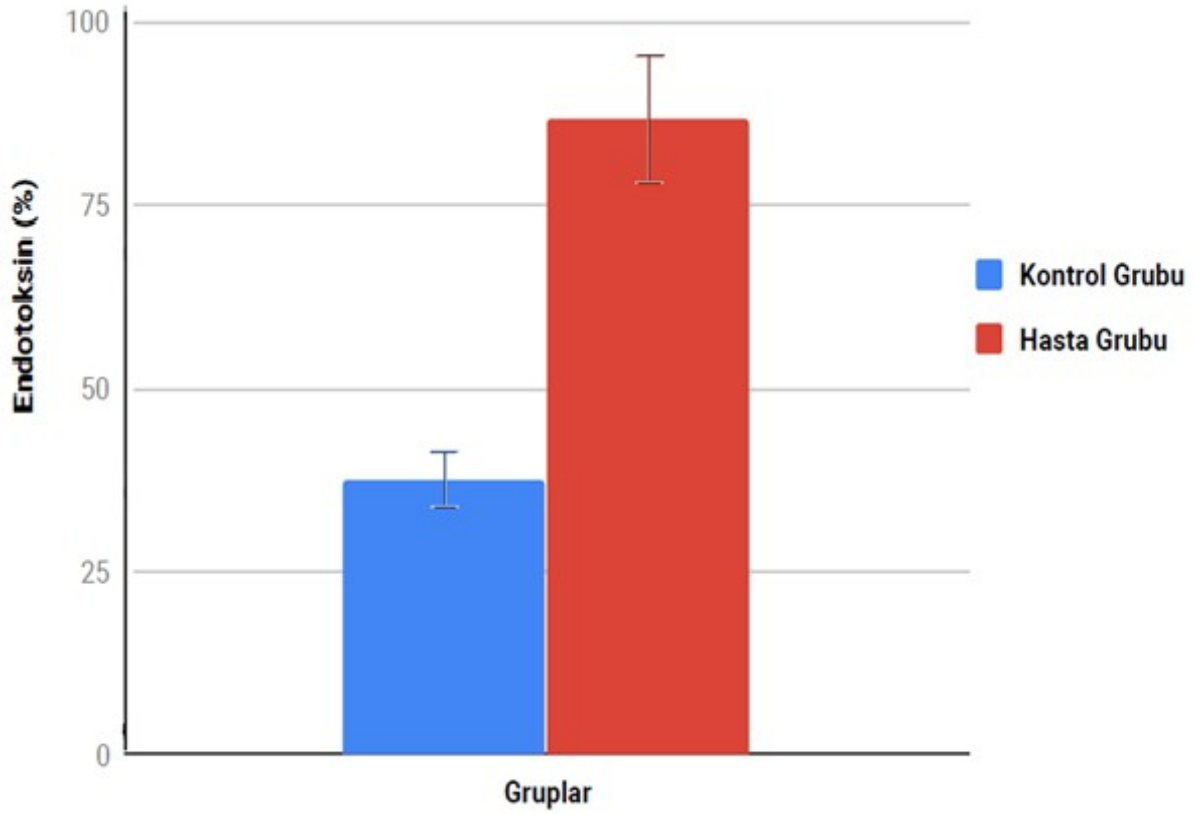

Figure 2: Comparison of serum endotoxin levels of H. pylori positive peptic ulcer patients and control groups. Serum endotoxin levels were significantly different between patients and controls ($p < 0.01$; Table 2).

Table -2: The means ± standard deviations of serum Endotoxin levels in H.pylori positive peptic ulcer patientsand control groups

Parameters	patient group(n=43)	Control group(n=43)	P value
Endotoxin (ng/ml)	10.39 ± 3.36 4	4.52±1.73	p<0.01

Pearson correlation analysis was performed between serum 8-OHdG and endotoxin values of patients with H. pylori positive peptic ulcer. DNA with potential for damage marker, there was a weak negative correlation between 8-OHdG and endotoxin levels (r = -0.259; Table 3).

Table-3: Pearson correlation analysis

Correlation	Endotoxin	N(Number)
8-OHdG-DNA Damage	r:-0,259	43 Patients 43 control

4. DISCUSSION

Infection by H. pylorioccurs worldwide. Infection first takes pplace in childhood and persists lifelong. Age and low socioeconomic level are main risk factors2. Its prevalence generally varies by ethnic group and geoFigureic regions2. Its prevalence in adults is 50–90% in developing countries and between 30 and 50% in developed countries (20-24).Hp leads to production of highly reactive molecules such as ROS by leukocytes and epithelial cells in gastric mucosa had role a role in creation of oxidative stress25. ROS are probably responsible for toxic actions on DNA resulting in formation of oxidized guanine(8-OHdG). The level of 8-OHdG can display the degree of DNA damage.Thuspersistent oxidative stress can induce a

chronic inflammation and peptic ulcer formation. Apart from gastric cancer, high levels of 8-OHdG were detected in a variety of diseases such asatherosclerosis, chronic obstructive pulmonary disease, diabetes18,26.

Increases in serum 8-OHdG levels have been reported in patients with H. pylori positive chronic gastritis, acute myocardial infarction and in type II diabetes mellitus among patients with Helicobacter pylori infection27,28.Although determination of urinary 8-OHdG is valuable in other diseseses such sytemic lupus eryth and in animal model of inflammatory bowel diseases, it is not useful for evaluation of the DNA damage in H. pylori-infected gastric mucosa in children29,30.In our findings, when serum 8-OHdG level reflecting DNA damage level was compared, a statistically significant difference was found between patients with H. pylori positive peptic ulcer and control groups ($p < 0.01$; Table 1).Therefore,8-OHdG functions is a sign of oxidative stress, leading to oxidative damage of DNA by reactive oxygen11. DNA damage interferes with DNA metabolic processes such as transcription and replication and can be powerful inhibitors of cell division and gene expression. A number of DNA repair mechanisms have been developed for genome stability, and when DNA lesions fall behind due to defects in the repair path, it changes the genetic information of the cell and mutations occur. Genome stability is essential for DNA repair, and defects in all major repair pathways can lead to cancer susceptibility31.

The other important finding is a significant but negatively weak relationship between plasma 8-OHdG and endotoxin. It was observed that H.pylori positive patients were associated with high endotoxin levels. It was suggested that endotoxin could be a potential candidate for inflammatory7.In our study, endotoxin levels in the H pylori group (10.39 ± 3.36) were found to be statistically significantly higher ($p < 0.01$) compared to the control group (4.52 ± 1.73). In study conducted by Ock CY et al, it was found high in patients with H pylori and patients with an active period7. İt was also reported that H.pylori infection in the gastric mucosa included oxidative DNA damage leading to cell cycle arrest and apoptosis and 8-OHdG analyzed immunohistochemically and showed 8-OHdG residue to confirm that H.pylori infection caused oxidative DNA damage. In our study, it was observed that there was a statistically significant difference between the patient and control groups for 8-OHdG, a metabolic product of DNA damageand for endotoxin analysis.

Conclusions: Infilteration of inflammatory cells in gastrointetinal tissues as a result of H. pylori infection. The sequalae is production of ROS and DNA damage. Persistent H pylori infection results in chronic gastritis, peptic ukcer and even cancers. 8-OHdG is a strong indicator of DNA damage due to oxidative stress that displays the risk of H. pylori disease. The increase in 8-OHdG values suggests its role of as a marker of H pylori gastrointestinal diseases. An increase in serum OHdG levelsmay be good biomarkers for presence of peptic ulcer due to H. pylori. These results suggest that it might be possible to determine H.pylori infection to some extent by determining a patient's serum 8−OHdG level. H.pylori and its endotoxin plays a significant role in the pathogensis of gastritis, peptic ulcers and gastric cancers through oxidative stress and DNA damages.

Conflict of interest statement The authors declare that there is no conflict of interest.

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