RESEARCH ARTICLE

Serum 8 Hydroxydeoxyguanosine and Cytotoxin Associated Gene A as Markers for *Helicobacter pylori* Infection

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Abstract

Background: *Helicobacter pylori* (*H.pylori*) is associated with chronic gastritis, peptic ulcers, gastric adenocarcinomas and mucosa associated tissue lymphomas. Cytotoxin associated gene A (CagA) is one of the virulence factors of *H.pylori*. It is hypothesized that reactive oxygen species (ROS) play roles in *H.pylori* associated disease especially in development of gastric adenocarcinoma. Individuals infected with *H.pylori* bearing CagA produce more ROS than others. 8-hydroxydeoxyguanosine (8OHdG) is an *in vitro* marker of DNA damage and oxidative stress. The aim of this study was to investigate the relationship between 8OHdG level, *H.pylori* infection and CagA and alterations of serum 8OHdG level after *H.pylori* eradication. Materials and Methods: Patients admitted with dyspeptic complaints and upper gastrointestinal endoscopy were assessed. *H.pylori* was determined from histopathology of specimens. Serum 8OHdG levels of three groups (*H.pylori* negative, *H. pylori* positive CagA negative and *H.pylori* positive CagA positive) were compared. Patients with *H.pylori* infection received eradication therapy. Serum 8OHdG levels pretreatment and posttreatment were also compared. Results: In total, 129 patients (M/F, 57/72) were enrolled in the study. Serum 8OHdG level of *H.pylori* negative, *H. pylori* positive CagA negative and *H.pylori* positive CagA positive groups were significantly different (5.77±1.35 ng/ml, 5.43±1.14 ng/ml and 7.57±1.25 ng/ml respectively, p=0.05). Furthermore, eradication therapy reduced serum 8OHdG level (6.10±1.54 ng/ml *vs* 5.55±1.23 ng/ml, p=0.05). Conclusions: Individuals infected with *H.pylori* bearing CagA strains have the highest serum 8OHdG level and eradication therapy decreases the serum 8OHdG level. To the best of our knowledge this is the first study that evaluated the effect of CagA virulence factor on serum 8OHdG level and the effect of eradication therapy on serum 8OHdG levels together. Eradication of CagA bearing *H.pylori* may prevent gastric adenocarcinoma by decreasing ROS. 8OHdG level may thus be a good marker for prevention from gastric adenocarcinoma

Keywords: *Helicobacter pylori* - CagA - eradication therapy

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Introduction

Helicobacter pylori (*H.pylori*) is one of the most common infections in humans. *H.pylori* is a gram negative microaerophilic microorganism. It colonises the gastric mucosa selectively and is known to be the major etiologic factor for chronic gastritis, peptic ulcer, gastric adenocarcinoma and mucosa associated tissue lymphoma (MALTOMA) (Moss and Calam, 1992; Ernst and Gold, 2000). Gastric cancer is the fifth most common cancer and third in cancer related death in all over the world (Ferlay et al., 2015). Various factors play role in development of peptic ulcer and gastric adenocarcinoma. Host and environmental factors also *H.pylori*'s virulence factors affect this process. It was demonstrated that *H.pylori* bearing cytotoxin associated gene A (CagA) increases the risk of ulcer and adenocarcinoma more than *H.pylori* without CagA (Peek, 2002).

Cells of aerobic organisms produces reactive oxygen species (ROS) during their metabolic and biochemical processes. ROS are essential for normal physiologic activity but its reactive nature can damage all parts of cells (Kehrer, 1993). Oxidative stress is a condition that arises from an imbalance between production of ROS and antioxidant defense system. In the last decades, oxidative stress was proposed as one of the underlying mechanisms of chronic illness that causes health and economic burden like diabetes mellitus, cardiovascular disease and cancer pathogenesis (Hussain et al., 2003; Margaret et al., 2011).

H.pylori infection recruits polymorphonuclear cells to the gastric mucosa and triggers inflammatory process. It was demonstrated that *H.pylori* infected gastric mucosa shows increased ROS and inflammatory markers (Noorgaard et al., 1995). ROS can also cause damage in the structure of DNA but these damages could be repaired. Excessive amount of unrepaired DNA can contribute to the

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pathogenesis of various types of cancer including gastric adenocarcinoma (Murata et al., 2012).

8 hydroxy-2-deoxyguanosine (8OHdG) is one of the products of DNA damage that is generated by reaction of guanosine with oxygen radical (OH-) and electron reduction (Kasai, 1997). This metabolic product can be measured by enzymatic methods in tissues and plasma and can be an indirect marker of oxidative DNA damage (Lunec et al., 2002).

Previous studies showed the association of 8OHdG, *H.pylori* infection and virulence factor (Everett et al., 2002; Farinati et al., 2003; Ladeira et al., 2004; Khodaii et al., 2011). Also it is demonstrated that *H.pylori* eradication therapy decreases 8OhDG level (Hahm et al., 1997; Nishibayashi et al., 2003; Katsurahara et al., 2009). But no study evaluate the 8OHdG level, CagA status and effect of eradication therapy on serum 8 OHdG together. The aim of this study was to investigate the relationship between serum 8OHdG level, *H.pylori* infection and CagA status. Also alterations of serum 8OHdG level after *H.pylori* eradication was evaluated.

Materials and Methods

Patients admitted to Gastroenterology Clinic and underwent esophago-gastroduodenal endoscopy were assessed for enrollment. Local ethical committee approved the study protocol and all patients gave informed content. (Ethics Committee approval no: 05 date: 03/15/2012).

Patients between 18 and 65 years of age were assessed for enrollment into the study. Patients with gastric surgery, with cholecystectomy, liver failure, renal failure, cardiac failure, malignancy, chronic obstructive pulmonary disease, diabetes mellitus, cerebrovascular disease, collagen tissue disease were excluded. Patients' medical history, liver function tests, kidney function tests, blood glucose tests and physical examination results were used together for diagnosis of exclusion criterias. Patients who received antibiotics, histamine 2 receptor antagonist or proton pump inhibitor in the past two weeks and patients with alcohol and tobacco consumption were excluded too.

During endoscopy, biopsy samples from antrum and corpus were taken for histopathologic examination and *H.pylori* diagnosis. Histopathologic assessment was made by Hematoxylin and Eosin stain in accordance with Sydney Classification. *H.pylori* was diagnosed by Giemsa stain.

Venous blood samples were drawn from all patients at the time of admission. ALT, AST, GGT, ALP, total protein, albumin, total bilirubin, direct bilirubin, total cholesterol, high density lipoprotein, low density lipoprotein, triglyceride, sedimentation, white blood cell, hemoglobine and platelets were measured within 30 minutes. Blood samples were centrifuged and stored at -80°C until assay. Serum 8OHdg levels and antibodies against CagA were measured with a commercial kit according to the manufacturer's instructions. (Japan Institute for the Control of Aging (JAICA)®, Shizuoka, Japan and DIA.PRO Diagnostic Bioprobes Srl®, Milano, Italy) respectively.

Serum 8 OHdG level were compared between *H.pylori* (+) and *H.pylori* (-) group.

Three groups were formed according to *H.pylori* and CagA status (*H.pylori* negative, *H.pylori* positive/CagA negative and *H.pylori*/CagA positive group) and serum 8 OHdG level were compared between these groups again.

Patients who diagnosed with *H.pylori* were received quadruple therapy including rabeprazol 20 mgr b.i.d., colloidal bismuth subcitrate 600 mg b.i.d., tetracycline 500 mg q.i.d. and metronidazole 500 mg t.i.d. for 14 days. 8 weeks after eradication therapy patients were assessed with C14 urea breath test (Heliprobe, Kibion AB Uppsala, Sweden). Patients with negative urea breath test were drawn blood samples again for 8OHdG measurement. Blood samples were centrifuged and stored at -80°C until assay.

SPSS version 15 was used for statistics. Continuous variables were reported as mean or median according to their homogeneity, categorical variables were reported as ratio. Distribution was assessed with Kolmogorov Smirnov test. Comparison of variables were done with Student's t test, Mann Whitney U test, Kruskal Wallis or $\chi 2$ test where applicable. Wilcoxon test was performed for comparison of pre and post treatment serum 8 OHdG levels. P value lower than 0.05 was considered statistically significant.

Results

One hundred twenty nine (129) patients [M/F, 57 (44.2%)/72 (55.8%)] were enrolled into the study. The mean age of patients was 36.8±10.9 years. The mean age of female and male patients were 36.1±11.3 years and 37.7±10.5 years ($p>0.05$).

Histopathological examination revealed that 94 (73%) patients had *H.pylori* infection, whereas 35 (27%) patients were *H.pylori* negative. Antibodies against CagA were analysed for 87 *H.pylori* positive patients. Seven patients were not included in analyse of antibody against CagA because of sampling errors or errors of laboratory analyses. Of these, 54 were positive for antibodies against CagA while the remaining 33 did not bear CagA strains. Demographic and clinical characters of *H.pylori* negative, *H.pylori* positive/CagA negative and *H.pylori*/CagA positive groups were shown in Table 1. There was no significant difference between *H.pylori* negative, *H.pylori* positive/CagA negative and *H.pylori*/CagA positive group with respect to gender, age (Table 1).

Serum 8OHdG levels of *H.pylori* (+) and *H.pylori* (-) group were similar (6.20±1.6 ng/ml *vs* 5.77±1.35 ng/ml, respectively $p>0.05$). Serum 8OHdG level of *H.pylori* negative, *H.pylori* positive/CagA negative and *H.pylori*/CagA positive groups were 5.77±1.35 ng/ml, 5.43±1.14 ng/ml, 7.57±1.25 ng/ml respectively. There are no statistical differences between *H.pylori* (-) and *H.pylori* (+) Cag A (-) group. ($p=0.154$) Patients infected with *H.pylori* bearing CagA strains have significantly higher 8 OHdG levels than other two groups (Figure 1) ($p<0.05$).

Eleven *H.pylori* infected patients were lost during follow-up period. Four patients could not receive therapy because of other comorbid situations appeared during follow-up. 79 patients received bismuth containing

Table 1. Demographic and clinical characters of groups according to *H.pylori* and CagA status

	H.pylori (-)	H.pylori (+) CagA (-)	H.pylori (+) CagA (+)	P value
Mean age, year	37.2±12.7	35.5±10.2	37.8±10.2	0.6
Female/male (number,% in total)	21/14 (17.2/11.5)	35/19 (28.7/15.6)	14/19 (11.5/15.6)	0.1
Glucose (mg/dl)	89.74	92.46	88.9	0.43
White blood cell/μL	6837	7379	6718	0.15
Hemoglobine (g/dl)	13.9	16.5	14.7	0.38
Trombocyte /μL	224714	226389	237343	0.63
Sedimantation	15.3	11.9	9.2	0.95
AST (U/L)	29.7	19.7	24.5	0.59
ALT (U/L)	30.7	21.3	26.7	0.52
GGT (U/L)	22.5	19.2	22.7	0.9
ALP (u/L)	93.1	96.8	84.1	0.76
Albumin (mg/dl)	4.33	4.26	4.32	0.47
Total protein (mg/dl)	7.37	7.44	7.34	0.51
Total Biluribine (mg/dl)	1	0.83	0.89	0.61
Direct Biluribine (mg/dl)	0.21	0.18	0.18	0.53
Blood urea nitrogen (mg/dl)	26.1	26.6	25.4	0.97
Creatinine (mg/dl)	0.8	0.77	0.88	0.55
Total Cholesterole (mg/dl)	166	170	179	0.75
LDL (mg/dl)	100	103	109	0.65
HDL (mg/dl)	45	44.4	43.9	0.41
Trigliceryde (mg/dl)	115	109	138	0.28

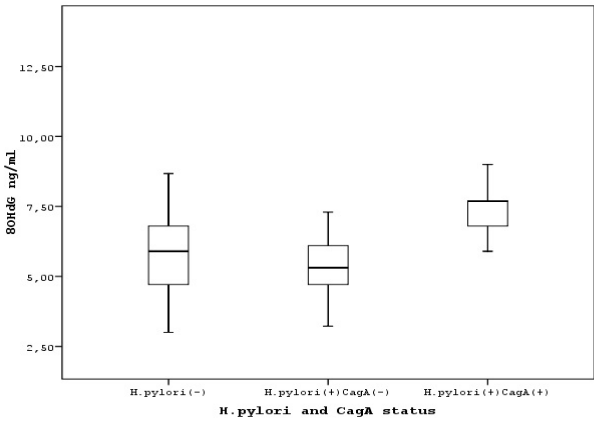

Figure 1. Comparison of Serum 8 OHdG Levels between Groups according to *H.pylori* and CagA Status

quadruple therapy. 5 patients discontinued due to adverse effects of therapy. 17 patients of whom received eradication therapy did not admit for control. 57 patients were performed urea breath test. 51 patients whom urea breath test were performed had negative urea breath test while 6 of them were positive. Eradication rate according to intention to treat (ITT) approach was 51/79 (64.5 %) while eradication rate according to per protocol (PP) approach was 51/57 (89%). Approximately half of the patients who received eradication therapy had complaints related to adverse effects.

Patients with negative urea breath test after eradication therapy were analysed for serum 8 OHdG level again. There was significant difference between pre treatment and post treatment serum 8 OHdG level. (6.10±1.54 ng/ml *vs* 5.55±1.23 ng/ml; respectively; p=0.004)

Discussion

The current study investigated the effect of *H.pylori* infection and CagA status on serum 8OHdG level. It was found that serum 8 OHdG level of patients without *H.pylori* and patients with *H.pylori* infection without CagA strains were similar. But patients with *H.pylori* infection bearing CagA strains had significantly higher level of 8 OHdG than other two groups. Also it was demostrated that eradication therapy decreased serum level 8 OHdG.

The relationship between infection, inflammation and carcinogenesis is debate of matter. *H.pylori* infection colonises antral mucosa and triggers inflammation. IL-8 related inflammation on gastric mucosa increases ROS production and increase in ROS production causes DNA damage (Naito and Yoshikawa, 2002). Also it was demonstrated that ROS production load is correlated with DNA damage load (Davies et al., 1994).

H.pylori causes proliferation and apoptosis in gastric mucosa. Carcinogenesis in gastric mucosa is related to alterations in gastric cell turnover rates (Jang and Kim, 2000). Proliferaton is not carcinogenic itself but makes gastric mucosa sensitive to mutagenic effects like ROS related DNA damage (Ames and Gold, 1990). Apoptotic cells increase in the infected gastric mucosa. Apoptotic cells are very rare in non infected mucosa and these cells significantly decrease after eradication therapy (Jang and Kim, 2000). Alterations in the cell turnover cause ulcer development and during ulcer healing, fibrosis and less differentiated cells take place normal gastric epithelial cells which can progress to intestinal metaplasia. A study demonstrated a mithochondria related apoptosis pathway in *H.pylori* infected gastric cells. In this study, ROS increased in infected gastric cells and adding vitamin E decreased ROS. ROS induced apoptosis alters the cell turnover rate and may cause carcinogenesis (Calvino Fernandez and Parra Cid, 2010).

Relationship between ROS and gastric carcinogenesis was demonstrated in a study that assesses the polymorphism of manganese superoxide dismutase (MnSOD). MnSOD involves in ROS scavengering and is a major antioxidant.

Polymorphism of MnSOD causes decreasing level of this enzyme. This study revealed a positive association bertween MnSOD mutation and gastric cancer risk (Moradi et al., 2015).

H.pylori may cause different clinical symptoms because of virulence factors of *H.pylori* especially CagA. It was demonstrated that *H.pylori* strains bearing CagA causes more serious inflammation on gastric mucosa and is associated with gastric carcinoma more than *H.pylori* without CagA strains. CagA bearing *H.pylori* strains code proteins that increase proinflammatory cytokines by stimulating gastric epithelial cells (Figura et al., 2000).

8 OHdG that is an *in vivo* marker of oxidative DNA damage that can be measured from tissues and plasma is increased in gastric mucosa infected with *H.pylori* (Everett et al., 2002; Ladeira et al., 2004). Besides correlation between levels of DNA damage and intensity of *H.pylori* gastritis was observed (Ladeira et al., 2004).

One study evaluated the relationship between 8OHdG and *H.pylori* CagA status. Gastritis scoring system was performed in that study. Higher gastritis scores, mononuclear activation, intestinal metaplasia and atrophy were associated with CagA status. Gastric mucosa infected with CagA bearing *H.pylori* had the highest level of 8 OHdG (Farinati et al., 2003). Our study reiterated those findings. Patients infected with CagA bearing *H.pylori* strains had significantly higher 8 OHdG levels than the patients with *H.pylori* negative and *H.pylori* without CagA strains in our study.

Our study did not find any significant difference between patients with and without *H.pylori* with respect to 8 OHdG. Another study demonstrated a significant difference but this study enrolled patients with gastric ulcer, duodenal ulcer and chronic gastritis and functional dyspepsia (Khodaii et al., 2011). In this study, the prevalence of CagA positivity was 70% among *H.pylori* positive patients. In our study, patients with duodenal ulcer, gastric ulcer and gastric carcinoma which were associated with CagA status were not included into the study so the prevalence was 35%. In this study association between serum level 8 OHdG and CagA status was not evaluated. Difference between patients with and without *H.pylori* may be explained by high prevalence of CagA in this study.

Another study reported controversial findings about association between 8 OHdG and *H.pylori* infection. In this study, 24 hour urine level of 8 OHdG was higher in *H.pylori* negative patients than the *H.pylori* positive ones. Also this study showed positive correlation with carbohydrate consumption and negative correlation with Vitamin C consumption (Witherell et al., 1998). *H.pylori* infection causes production of reactive nitrogen species besides ROS. 8-nitroguanine is an *in vivo* marker of reactive nitrogen species that can be produced during *H.pylori* infection and both 8 OHdG and reactive nitrogen species can contribute to gastric carcinoma pathogenesis (Ohnishi et al., 2013). One study evaluated the association between *H.pylori* infection, 8 OHdG and 8-nitroguanin. 8 OHdG and 8-nitroguanin in gastric mucosa were related to intestinal metaplasia, glandular atrophy and intensity of *H.pylori* infection. Also eradication of infection reduced the level of both 8 OHdG and 8 nitroguanin (Katsurahara et al., 2009). In our study also post eradication level of 8 OHdG were lower than the pre eradication level. Two other study confirmed our study's report about effect of eradication on 8 OHdG. Eradication therapy decreases the 8 OHdG level on gastric mucosa (Hahm et al., 1997; Nishibayashi et al., 2003).

Another study evaluated the combination of CagA gene expression and 8 OHdG in tissue samples of gastritis, gastric cancer and non-dyspeptic controls. This study demonstrated that CagA gene expression and high expression of 8 OHdG was correlated with severe gastric inflammation and gastric cancer (Raza et al., 2014). Also another study demonstrated that individuals with gastric cancer had higher levels of 8 OHdG than the control group (Ma et al., 2013)

Our study had some limitations. First of all, while patients with gastric ulcer, duodenal ulcer and chronic disease like diabetes mellitus, atherosclerosis which may contribute to serum 8 OHdG level were excluded from the study, diet factor was not taken into account. Also we measured 8 OHdG from serum, most of the studies mentioned above analysed 8 OHdG from gastric mucosa samples. *H.pylori* eradication therapy is such a treatment that can be found inconvient by patients. Only 57 of 94 *H.pylori* positive patients could be performed urea breath test. This situation may affect the difference between pre and post eradication 8 OHdG level.

Gastrointestinal tract is probably the one of the most important part of the organism because there is an continous interaction between materials such foods, microorganisms and gastrointerstinal tract. Most of the diseases' initiation and progression can be related to gastrointestinal tract dysfunction or carcinogenic materials that ingested by organisms. Excessive ROS of gastrointestinal tract due to antigens and microorganisms can be an intriguing issue for prevention and treatment of gastric cancer. *H.pylori* can be accepted as one of the reasons for excessive ROS of gastrointestinal tract

H.pylori infection contribution on inflammation and gastric carcinoma development may be due to CagA virulence factor. It is not clear how can *H.pylori* evoke inflammation and carcinoma development

In conclusion, CagA status affects serum level 8 OHdG more than *H.pylori* infection without CagA. Also eradication therapy decreases serum 8 OHdG level. Association between inflammation, *H.pylori* infection, carcinoma development must be evaluated with large number of patients and more oxidative stress markers. If sensitive biomarkers of oxidative stress related to gastric carcinogenesis can be found, gastric cancer can be a disease that can be prevented.

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