Urinary 8-Oxo-7,8-Dihydro-2′-Deoxyguanosine in Patients with Parasite Infection and Effect of Antiparasitic Drug in Relation to Cholangiocarcinogenesis

Raynoo Thanan,1,4,6 Mariko Murata,6 Somchai Pinlaor,2,4 Paiboon Sithithaworn,2,4 Narong Khuntikeo,3,4 Walaluk Tangkanakul,5 Yusuke Hiraku,6 Shinji Oikawa,6 Puangrat Yongvanit,1,4 and Shosuke Kawanishi6,7

Departments of ‘Biochemistry, ‘Parasitology, and ‘Surgery, ‘Liver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand; ‘Bureau of General Communicable Diseases, Department of Disease Control, Ministry of Public Health, Thailand; ‘Department of Environmental and Molecular Medicine, Mie University Graduate School of Medicine, Tsu, Mie, Japan; and ‘Faculty of Health Science, Suzuka University of Medical Science, Suzuka, Mie, Japan

Abstract

Parasite infection of Opisthorchis viverrini is a major risk factor for cholangiocarcinoma. Our previous immunohistochemical studies showed that O. viverrini infection induced oxidative DNA lesions in the bile duct epithelium during cholangiocarcinoma development. The current study assessed the levels of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), an oxidative DNA lesion, in the urine and leukocytes of O. viverrini–infected subjects and cholangiocarcinoma patients. Forty-nine O. viverrini–infected patients, 55 cholangiocarcinoma patients, and 17 healthy controls were enrolled in the study. We measured 8-oxodG levels in the urine and leukocytes of these subjects using an electrochemical detector coupled to high-performance liquid chromatography. O. viverrini–infected patients were assessed before treatment and 2 months and 1 year after praziquantel treatment. Urinary 8-oxodG levels were significantly higher in cholangiocarcinoma patients (6.83 ± 1.00 µg/g creatinine) than in O. viverrini–infected patients (4.45 ± 0.25 µg/g creatinine; P < 0.05) and healthy subjects (3.03 ± 0.24 µg/g creatinine; P < 0.01) and higher in O. viverrini–infected subjects than in healthy subjects (P < 0.01). The urinary 8-oxodG levels in O. viverrini–infected patients significantly decreased 2 months after praziquantel treatment and were comparable with levels in healthy subjects 1 year after treatment. Urinary 8-oxodG levels were significantly correlated with leukocyte 8-oxodG levels, plasma nitrate/nitrite levels, and aspartate aminotransferase activity. In conclusion, this study, in addition to our previous studies, indicates that 8-oxodG formation by parasite infection may play an important role in cholangiocarcinoma development. Urinary 8-oxodG may be a useful biomarker to monitor not only infection but also carcinogenesis. (Cancer Epidemiol Biomarkers Prev 2008;17(3):518–24)

Introduction

Chronic infection is well associated with some types of cancer through chronic inflammation (1-3). Large amounts of reactive oxygen species produced during inflammation are associated with an increased risk of human cancer (1, 2, 4). Oxidatively damaged DNA lesions have been suggested to contribute to several diseases, including cancers. 8-Oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), an accepted marker for reactive oxygen species–induced DNA damage (5), is a mutagenic lesion leading to G → T transversions (6, 7) that are frequently found in tumor-associated genes in a variety of cancers (8, 9). Furthermore, some reports have shown elevated levels of urinary 8-oxodG in patients with different types of cancer (10-12). Therefore, urinary 8-oxodG may be a useful biomarker for cancer initiation and/or progression in cases where inflammation contributes to carcinogenesis. Measuring urinary 8-oxodG is noninvasive and therefore is an acceptable assay to biomonitor exposure to environmental carcinogens (13), including infection.

Parasite infection of Opisthorchis viverrini is classified as a group 1 carcinogen by the IARC (14) and is a risk factor for cholangiocarcinoma in Southeast Asia (15). O. viverrini infestation is endemic in Khon Kaen province, northeastern Thailand, and Khon Kaen has the highest incidence of cholangiocarcinoma in the world with an 80% to 90% occurrence rate (16). Several studies have shown that O. viverrini infection induces inflammation in both animal models (17, 18) and humans (19). Our previous studies (17, 20) on a hamster model showed that chronic inflammation induced by O. viverrini infection is a potential risk factor for cholangiocarcinoma, raising the question whether O. viverrini infection and cholangiocarcinoma development are reflected by the levels of 8-oxodG secreted in the urine.

Received 10/18/07; revised 12/4/07; accepted 12/15/07.

Grant support: Thailand Research Fund through Royal Golden Jubilee Ph.D. Programme (R. Thanan and P. Yongvanit), Khon Kaen University Research Fund and Faculty of Medicine, and Ministry of Education, Culture, Sports, Science and Technology of Japan Grants-in-Aid for Scientific Research.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: P. Yongvanit and S. Kawanishi contributed equally to this work.

Requests for reprints: Shosuke Kawanishi, Faculty of Health Science, Suzuka University of Medical Science, Suzuka, Mie 510-0293, Japan. Phone: 81-59-381-2388; Fax: 81-59-381-2386. E-mail: kawanishi@suzuka-u.ac.jp

Copyright © 2008 American Association for Cancer Research.
doi:10.1158/1055-9965.EPI-07-2717

Cancer Epidemiol Biomarkers Prev 2008;17(3). March 2008
To evaluate the usefulness of 8-oxodG as a factor to predict the risk of cholangiocarcinoma in *O. viverrini*–infected individuals, we measured 8-oxodG levels in the urine and leukocytes of healthy controls, *O. viverrini*–infected subjects, and cholangiocarcinoma patients using high-performance liquid chromatography coupled with an electrochemical detector. The effect of praziquantel, an effective drug for *O. viverrini* egg count in stool, and the average age was 48.43 ± 13.11 years. Liver flukes were eliminated in the pretreated *O. viverrini*–infected group by praziquantel (a single dose of 40 mg/kg body weight).

Two months and 1 year after praziquantel treatment, patients whose stool was negative for *O. viverrini* eggs were defined as the posttreatment group. Obese, diabetic, and patients with chronic inflammation diseases, such as hepatitis virus and tuberculosis infections, were excluded from the study. Cholangiocarcinoma subjects (38 males and 17 females) were defined as patients who underwent surgical resection for cholangiocarcinoma at the Department of Surgery, Faculty of Medicine, Khon Kaen University, and this group had an average age of 54.02 ± 10.18 years. Cholangiocarcinoma was verified by histopathologic examination; other inflammatory diseases, such as cholangitis and hepatocarcinoma, were not included in this study. There was no significant difference in the average age of *O. viverrini*–infected subjects and healthy control subjects, but the average age of cholangiocarcinoma patients was higher than the other groups.

**Subjects and Methods**

**Subjects.** The protocol for this study was approved by the Ethics Group of the Human Research Committee (HE480316), Khon Kaen University, Thailand. The subjects in this study were divided into three groups: healthy subjects, *O. viverrini*–infected patients, and *O. viverrini*–infected cholangiocarcinoma patients. All participants volunteered for this study and gave informed consent. Both healthy control and *O. viverrini*–infected groups live in Khon Kaen province and have similar lifestyles, including smoking and alcohol drinking, although healthy control subjects have no habit of eating raw freshwater fishes, which transmit *O. viverrini*. Cholangiocarcinoma patients in hospital had no current alcohol drinking and smoking. Healthy subjects (8 males and 9 females) had an average age of 47.70 ± 8.01 years and were defined as persons who had never been infected with *O. viverrini*. Pretreated *O. viverrini*–infected patients (22 males and 27 females) had a positive *O. viverrini* egg count in stool, and the average age was 48.43 ± 13.11 years. Liver flukes were eliminated in the pretreated *O. viverrini*–infected group by praziquantel (a single dose of 40 mg/kg body weight).

**Specimen Collection and Storage.** First morning urine specimens were collected, immediately frozen, and maintained at −80°C until analysis. After at least 12 h of fasting, peripheral blood was obtained by stabilized venipuncture, collected in tubes (10 mL/tube) with acid citrate dextrose, and centrifuged at 2,000 rpm at 4°C for 15 min, and the Buffy coat and plasma were isolated. The Buffy coat was treated with RBC lysis buffer (10 mmol/L Tris-HCl (pH 7.6), 5 mmol/L MgCl₂, and 10 mmol/L NaCl) to remove RBC and obtain a WBC pellet. WBC pellets were washed twice with sterile PBS. WBC pellets and plasma were stored at −80°C until use.

**Measurement of 8-oxodG in Urine.** 8-oxodG levels in the urine were determined by the method of Kasai et al. with slight modifications (21). Briefly, frozen samples were thawed and mixed completely. Urine samples were checked by Uropaper III (Eiken Kagaku K.K.) to exclude samples containing abnormal levels of WBC, RBC/hemoglobin, ketone, glucose, and protein. Thereafter, urine was centrifuged at 1,500 rpm for 5 min at 4°C, and the supernatant was transferred to a vial with 8-hydroxyguanosine (Cayman Chemical), a ribonucleoside marker. A 20 μL sample was injected into the high-performance liquid chromatography column (MCI GEL CA08F, 7 μm, 1.5 × 150 mm) at 58°C with a flow rate of 0.045 mL/min with the mobile phase consisting of 3 mmol/L H₂SO₄ and 2% (v/v) acetonitrile. The chromatogram was monitored by a UV detector (254 nm) depending on the relative elution position of the added marker to collect the 8-oxodG fraction. This fraction was injected into the second high-performance liquid chromatography (a Capcell Pak C18 column, 5 μm, 4.6 × 150 mm; Shiseido) at 30°C and a flow rate of 1 mL/min with the mobile phase consisting of 10 mmol/L NaH₂PO₄ and 5% (v/v) methanol. The 8-oxodG content was analyzed using an electrochemical detector (Coulormem II 5200A, ESA Biosciences) with the electrode voltage set to +300 mV. The molar ratio of 8-oxodG in each sample was measured based on the peak height of authentic 8-oxodG (Calbiochem) by the electrochemical detector. The urinary concentration of 8-oxodG was corrected by dividing the individual urinary creatinine concentrations. Urinary creatinine concentrations were determined using a creatinase method (Wako Pure Chemical Ind.).

**Measurement of 8-oxodG in Leukocyte DNA.** DNA was extracted from WBC pellets with a DNA Extractor WB Kit (Wako Pure Chemical Ind.). 8-oxodG levels in DNA were measured as described previously with slight modifications (22). DNA was dissolved in 20 mmol/L acetic acid buffer (pH 5.0) and digested to deoxynucleosides by incubating with 4 units nuclease P₁ (Wako Pure Chemical Ind.) at 37°C for 30 min followed by 0.6 units bacterial alkaline phosphatase (Sigma Chemical) at 37°C for 1 h in 0.1 mol/L Tris-HCl (pH 7.5). The deoxynucleosides were analyzed using electrochemical detector (Coulormem II 5200A, ESA Biosciences) coupled with high-performance liquid chromatography equipped with a Capcell Pak C18 column (4.6 × 150 mm; Shiseido). The mobile phase consisted of 100 mmol/L NaH₂PO₄ and 5% (v/v) methanol. The 8-oxodG content was measured based on the electrochemical detector peak height of authentic 8-oxodG and the UV absorbance of 2′-deoxyguanosine at 254 nm.

**Analysis of Plasma Nitrate/Nitrite and AST.** The plasma levels of nitrate and nitrite were determined by the vanadium-based simple spectrophotometric method.
using the Griess reaction as described previously by Miranda et al. (23) with a minor modification. The assay was done in a standard flat-bottomed 96-well polystyrene microtiter plate. Nitrate concentrations in biological samples were measured after reducing to nitrite with the catalyst, VCl₃. Plasma (100 µL) was deproteinized to reduce turbidity with 200 µL cold absolute methanol/ diethyl ether (3:1, v/v) for 30 min at −80 °C. The samples were centrifuged at 12,000 × g for 10 min, and the supernatant was analyzed for nitrate and nitrite. After 100 µL supernatant or standard nitrite was added to each well, 100 µL VCl₃ was added followed by the immediate addition of 100 µL of the Griess reagents [premixed 50 µL of 2% sulfanilamide in 5% HCl and 50 µL of 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride]. The contents were vigorously mixed and the plate was incubated for 20 min at 37 °C. The 540 nm absorbance was measured to assess the total amounts of nitrate and nitrite using sodium nitrite as the standard.

Plasma AST activities were determined spectrophotometrically using an automated analyzer.

Statistical Analysis. Data and transformed log-scale data were tested for data distribution. One-way ANOVA and Kruskal-Wallis tests were used to determine statistical significance in the normal and nonnormal distribution of data among cholangiocarcinoma patients, O. viverrini–infected patients, and healthy control groups. Significant differences between groups were analyzed by a Student’s t test or Mann-Whitney’s U test for normal distribution and nonnormal distribution data, respectively. The individual O. viverrini–infected patients were analyzed by the Friedman test and Wilcoxon signed-rank test to compare the degree of parasitic infection between the pretreatment and post-treatment groups at a 2-month and 1-year follow-up visit. Spearman’s rank correlation coefficient was used to analyze correlations for nonparametric data, whereas Pearson’s correlation coefficient was used for parametric data. P values less than 0.05 were considered statistically significant.

Results

Figure 1 shows urinary 8-oxodG levels in healthy, O. viverrini–infected, and cholangiocarcinoma subjects. The levels of urinary 8-oxodG in cholangiocarcinoma patients (6.83 ± 1.00 µg/g creatinine) were significantly higher than those in O. viverrini–infected patients (4.45 ± 0.25 µg/g creatinine; P < 0.05) and healthy subjects (3.03 ± 0.24 µg/g creatinine; P < 0.01; Fig. 1A). The urinary 8-oxodG levels in O. viverrini–infected patients were significantly higher than those in healthy subjects (P < 0.01). Figure 1B shows the effect of praziquantel treatment on 8-oxodG levels in O. viverrini–infected and healthy subjects. Two months after treatment, there was no significant difference between pretreated patients and healthy subjects. The urinary 8-oxodG levels in treated patients 1 year after therapy were significantly lower than those in pretreated patients (P < 0.01) and near levels in healthy control subjects. Figure 1C shows the time course of urinary 8-oxodG levels in O. viverrini–infected patients who were monitored 2 months and 1 year after O. viverrini removal treatment. 8-oxodG

---

**Figure 1.** Urinary 8-oxodG levels in cholangiocarcinoma patients, O. viverrini–infected patients, and healthy control subjects. A. Urinary 8-oxodG levels in healthy (H; n = 15), O. viverrini–infected patients (OV; n = 49), and cholangiocarcinoma patients (CCA; n = 29). ***, P < 0.01, compared with healthy subjects; #, P < 0.05; compared with O. viverrini–infected patients. B. Effect of praziquantel on urinary 8-oxodG levels in the O. viverrini–infected group [OV, pretreatment (n = 49); 2M, 2 months posttreatment (n = 34); 1Y, 1 year posttreatment (n = 21)]. ***, P < 0.01, compared with healthy subjects; ###, P < 0.01, compared with pretreated O. viverrini–infected patients. C. Time course of urinary 8-oxodG levels in O. viverrini–infected subjects treated with praziquantel. Urinary 8-oxodG levels were assessed in individual O. viverrini–infected subjects (n = 14), who could be followed for 1 year. #, P < 0.05; ###, P < 0.01, compared with pretreatment; $, P < 0.05; compared with 2 months after treatment. Mean ± SE.
levels in the urine gradually improved after treatment and were significantly decreased 2 months ($P < 0.05$) and 1 year ($P < 0.01$) after treatment.

Figure 2 shows leukocyte 8-oxodG levels in healthy, *O. viverrini*–infected, and cholangiocarcinoma subjects. Leukocyte 8-oxodG levels in cholangiocarcinoma patients ($1.18 \pm 0.22/10^5$ dG) were significantly higher than those in healthy subjects ($0.30 \pm 0.02/10^5$ dG; $P < 0.01$). Although a similar trend was observed with urinary 8-oxodG, the level of leukocyte 8-oxodG was not significantly different ($P = 0.052$) between *O. viverrini*–infected subjects ($0.97 \pm 0.10/10^5$ dG) and cholangiocarcinoma patients (Fig. 2A). The levels of leukocyte 8-oxodG 2 months ($P < 0.01$) and 1 year ($P < 0.05$) after *O. viverrini* removal were significantly lower in the *O. viverrini*–infected group, but both levels were still significantly ($P < 0.01$) higher than levels in healthy control subjects. The time course of leukocyte 8-oxodG levels gradually decreased but was not significantly different by the Friedman test (Fig. 2C).

Figure 3 shows plasma nitrate/nitrite levels in healthy, *O. viverrini*–infected, and cholangiocarcinoma subjects. The plasma nitrate/nitrite levels in *O. viverrini*–infected and cholangiocarcinoma patients were significantly ($P < 0.01$) higher than those in healthy subjects (Fig. 3A). However, there was no significant difference between *O. viverrini* infection and cholangiocarcinoma. Two months after treatment, the plasma levels of nitrate/nitrite were significantly ($P < 0.01$) lower than those in pretreated subjects and reached levels similar to those in healthy subjects (Fig. 3B). Plasma nitrate/nitrite levels were significantly decreased 2 months ($P < 0.05$) and 1 year ($P < 0.01$) after *O. viverrini* eradication by praziquantel (Fig. 3C).

Leukocyte 8-oxodG levels in cholangiocarcinoma patients, *O. viverrini*–infected patients, and healthy control subjects. A. Leukocyte 8-oxodG levels in healthy controls ($n = 15$), *O. viverrini*–infected patients ($n = 32$), and cholangiocarcinoma patients ($n = 55$). **, $P < 0.01$, compared with healthy subjects; #, $P < 0.05$; ##, $P < 0.01$, compared with healthy subjects. B. Effect of praziquantel on leukocyte 8-oxodG levels in the *O. viverrini*–infected group [OV, pretreatment ($n = 32$); 2M, 2 months posttreatment ($n = 31$); 1Y, 1 year posttreatment ($n = 34$)]. **, $P < 0.01$, compared with healthy subjects; #, $P < 0.05$; ##, $P < 0.01$, compared with pretreated *O. viverrini*–infected patients. C. Time course of leukocyte 8-oxodG levels in *O. viverrini*–infected subjects treated with praziquantel. The leukocyte 8-oxodG levels were assessed in individual *O. viverrini*–infected subjects ($n = 16$) who could be followed for 1 year. Mean $\pm$ SE.

**Discussion**

The present study was the first to show that 8-oxodG levels in urine and isolated leukocyte DNA were...
after treatment. Mean

\( P \) compared with pretreated

\( O. \) viverrini

\( P \) \(<\) 0.01, compared with healthy subjects; ##,

ment (\( n \))

\( O. \) viverrini

compared with healthy subjects.

\( B. \)

\( n \)–infected patients (\( n = 37 \)). **, \( P \) \(<\) 0.01, compared with healthy subjects. B. Effect of praziquantel

on plasma nitrate/nitrite levels in the \( O. \) viverrini–infected group [\( OV \), pretreatment (\( n = 32 \)); 2M, 2 months posttreatment (\( n = 31 \)); 1Y, 1 year posttreatment (\( n = 34 \))]. **, \( P \) \(<\) 0.01, compared with healthy subjects; ##, \( P \) \(<\) 0.01, compared with pretreated \( O. \) viverrini–infected patients. C. Time course of plasma nitrate/nitrite levels in \( O. \) viverrini–infected patients treated with praziquantel. The plasma nitrate/nitrite levels were assessed in individual \( O. \) viverrini–infected subjects (\( n = 11 \)), who could be followed for 1 year. #, \( P \) \(<\) 0.05; ##, \( P \) \(<\) 0.01, compared with pretreatment; $, \( P \) \(<\) 0.05; compared with 2 months after treatment. Mean ± SE.

![Figure 3](image-url)

Figure 3. Plasma nitrate/nitrite levels in cholangiocarcinoma patients, \( O. \) viverrini–infected patients, and healthy control subjects. A. Plasma nitrate/nitrite levels in healthy controls (\( n = 13 \)), \( O. \) viverrini–infected patients (\( n = 43 \)), and cholangiocarcinoma patients (\( n = 37 \)). **, \( P \) \(<\) 0.01, compared with healthy subjects. B. Effect of praziquantel on plasma nitrate/nitrite levels in the \( O. \) viverrini–infected group [\( OV \), pretreatment (\( n = 32 \)); 2M, 2 months posttreatment (\( n = 31 \)); 1Y, 1 year posttreatment (\( n = 34 \))]. **, \( P \) \(<\) 0.01, compared with healthy subjects; ##, \( P \) \(<\) 0.01, compared with pretreated \( O. \) viverrini–infected patients. C. Time course of plasma nitrate/nitrite levels in \( O. \) viverrini–infected patients treated with praziquantel. The plasma nitrate/nitrite levels were assessed in individual \( O. \) viverrini–infected subjects (\( n = 11 \)), who could be followed for 1 year. #, \( P \) \(<\) 0.05; ##, \( P \) \(<\) 0.01, compared with pretreatment; $, \( P \) \(<\) 0.05; compared with 2 months after treatment. Mean ± SE.

Interestingly, 8-oxodG levels in the urine were significantly higher in cholangiocarcinoma patients than in \( O. \) viverrini–infected subjects. The higher level of 8-oxodG in cholangiocarcinoma patients suggests that there is additional oxidative stress in cancer tissue and that urinary 8-oxodG may reflect a systemic burden both during \( O. \) viverrini infection and cholangiocarcinoma development. These results also indicate that urinary 8-oxodG may be a potential biomarker for predicting the risk of cholangiocarcinoma development.

Urinary 8-oxodG levels were significantly and positively correlated with plasma nitrate/nitrite levels. The accumulation of oxidative DNA damage may be associated with the production of reactive oxygen and nitrogen species in \( O. \) viverrini infection. Superoxide anion radical \((O_2^-)\) is derived from eosinophils (27) and macrophages (28) and dismutated to \( H_2O_2 \), which induces metal-dependent 8-oxodG formation (29).

Our previous results showed that \( O. \) viverrini–infected hamsters had higher inducible NO synthase levels and increased 8-oxodG formation in the epithelial bile duct and in inflammatory cells at the site of infection (17, 30). In addition, inducible NO synthase expression in both inflammatory cells and bile duct epithelium was closely related with plasma nitrate/nitrite levels. The end products of NO (30).

It is well known that NO reacts with \( O_2^- \) to produce peroxynitrite (ONOO\(^-\)), which also can induce 8-oxodG (31). Notably, a longitudinal observation of \( O. \) viverrini–infected individuals showed that plasma levels of nitrate/nitrite and 8-oxodG gradually decreased 2 months after parasite treatment and reached levels found in healthy control subjects after 1 year.

Our previous immunohistochemical analyses showed that 8-oxodG in the biliary epithelium of \( O. \) viverrini–infected hamsters resulted from the inflammatory response because 8-oxodG levels decreased after praziquantel treatment (32). A single praziquantel dose can eradicate \( O. \) viverrini as measured by egg counts in the stool; however, some inflammation with oxidative stress continued in the treated patients. Additional clinical approaches, such as anti-inflammation and antioxidative

...
strategies, may be required for several months after 
O. viverrini treatment.

It is known that the feeding and migrating activities of 
flukes contribute to hepatobiliary injury (33). In addition, 
immunopathologic processes may contribute to long-
term hepatobiliary damage through O2− and NO gene-
rated from eosinophils and activated macrophages, 
resulting in 8-oxodG formation. In this study, urinary 
8-oxodG levels positively correlated with AST activity in 
healthy subjects, O. viverrini–infected patients, and 
cholangiocarcinoma patients. The average AST level 
was significantly higher in O. viverrini–infected patients 
than healthy subjects but still within the normal range.
AST activity in the plasma of cholangiocarcinoma 
patients was much higher than that in healthy subjects. 
This result is supported by a previous report show-
ing that the number of 8-oxodG-positive hepatocytes 
was associated with AST activity in liver diseases and 
hepatocellular carcinoma (34). These findings can be 
explained by assuming that an increase in reactive 
oxygen species not only causes DNA damage but also 
induces hepatocyte injury, resulting in increased hep-
atabiliary enzyme activity.

A series of previous studies on both animal models 
and humans indicate that O. viverrini infection can 
induce the accumulation of potentially mutagenic 
modifications via reactive oxygen species production 
at inflammation sites and that these DNA lesions ini-
tiate carcinogenesis. We first showed that O. viverrini– 
induced oxidative stress could be detected by 
8-oxodG that accumulates in leukocyte DNA and is 
excreted in the urine of O. viverrini–infected patients. 
Furthermore, 8-oxodG levels may increase during 
holangiocarcinoma development in O. viverrini–associ-
ated cholangiocarcinoma patients, whereas eradication 
of O. viverrini decreases 8-oxodG levels. In the light 
of the presented data, we conclude that 8-oxodG levels, 
especially in the urine, may be a useful biomarker 
not only to monitor O. viverrini infection but also to 
assess the risk of O. viverrini–associated cholangiocar-
cinogenesis.

Acknowledgments
We thank the volunteers who donated their samples for this 
study.

References
stress and p53 activation in chronic inflammation. Proc Natl Acad Sci 
860–7.
3. Ohshima H, Bartsch H. Chronic infections and inflammatory 
processes as cancer risk factors: possible role of nitric oxide in 
4. Dedon PC, Tannenbaum SR. Reactive nitrogen species in the 
chemical biology of inflammation. Arch Biochem Biophys 2004;423:
12–22.
moiety in cellular DNA by agents producing oxygen radicals and 
6. Bruner SD, Norman DP, Verdine GL. Structural basis for recognition 
and repair of the endogenous mutagen 8-oxoguanine in DNA. 
7. Shibutani S, Takeshita M, Grollman AP. Insertion of specific bases 
during DNA synthesis past the oxidation-damaged base 8-oxoguanine. 

Figure 4. Plasma AST activity in cholangiocarcinoma 
patients, O. viverrini–infected patients, and healthy control 
subjects. A. Plasma AST activity in healthy controls (n = 17), 
O. viverrini–infected patients (n = 47), and cholangiocarci-
noma patients (n = 49). **, P < 0.01, compared with healthy 
subjects; ##, P < 0.01, compared with O. viverrini–infected 
patients. B. Effect of praziquantel on the plasma AST activity 
in the O. viverrini–infected group [OV, pretreatment (n = 47); 
2M, 2 months posttreatment (n = 51); 1Y, 1 year posttreatment 
(n = 28)]. **, P < 0.01, compared with healthy subjects; 
#, P < 0.05, compared with pretreated O. viverrini–infected 
patients. C. Time course of plasma AST activity in 
O. viverrini–infected patients treated with praziquantel. 
The plasma AST levels were assessed in individual 
O. viverrini–infected subjects (n = 11), who could be followed 
for 1 year. Mean ± SE.