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Increased expression of TLR-2, COX-2, and SOD-2 genes in the peripheral blood leukocytes of opisthorchiasis patients induced by *Opisthorchis viverrini* antigen

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Abstract Re-infection with liver fluke, *Opisthorchis viverrini*, increases proinflammatory molecules involved in inflammation-mediated disease and carcinogenesis in an animal model. To clarify whether these genes respond to parasite antigen in peripheral blood leukocytes (PBL) of opisthorchiasis patients, we examined the transcriptional level of oxidant-generating (toll-like receptor 2 (TLR-2), nuclear factor-kappa B (NF-KB), and cyclooxygenase 2 (COX-2)), anti-oxidant-generating (manganese superoxide dismutase 2 (SOD-2) and catalase (CAT)), proinflammatory cytokine (interleukin (IL)-1β), and anti-inflammatory cytokine (IL-10), in PBL exposed to parasite antigen in *O. viverrini*-infected patients compared with healthy individuals in an in vitro experiment. After *O. viverrini* antigen-treated PBL, quantitative RT-PCR analysis revealed that increased expression of cytokines and oxidant-generating genes in PBL was similar between *O. viverrini*-infected and healthy groups. Interestingly, compared with healthy subjects, increase of TLR-2, COX-2, and SOD-2 and decreased CAT mRNA expression levels were observed in *O. viverrini*-infected group. The results indicate that *O. viverrini* antigen induces upregulation of TLR-2, COX-2, and SOD-2 and downregulation of CAT genes in opisthorchiasis patients, suggesting that imbalance of oxidant/anti-oxidant transcripts during re-infection may be involved in the inflammatory-driven carcinogenesis. These molecules may be used as the chemopreventive target for intervention of opisthorchiasis patients in an endemic area.

Introduction

The liver fluke, *Opisthorchis viverrini*, is classified as a group I carcinogen by the IARC, which is a relative risk factor for cholangiocarcinoma (CCA), in humans in Southeast Asia (IARC 1994). *O. viverrini* infection is endemic in northeastern Thailand, Khon Kaen Province, where the highest incidence of CCA in the world has been reported (Sriamporn et al. 2004). In Thailand, the estimated number of people infected with *O. viverrini* is eight million with an average of 9.6% of the population to be infected (Jongsuksuntigul and Imsomboon 2003). Mass treatment programs seem to decrease the burden of infection; however, re-infection more likely occurs after praziquantel treatment in endemic areas (Sripa et al. 2007; Saowakontha et al. 2000). Humans become infected with *O. viverrini* by consuming raw or undercooked fish that contain metacercaria, the infective stage. Adult worm lives in the biliary tract and produces eggs, which is excreted in the feces after 1 month post-infection. Most of the infection-induced chronic disease remains clinically silent unless actively detected by
ultrasound (Mairiang et al. 2006). Antigens released by adult worms stimulate inflammation around bile ducts not only in the large bile duct but also in the small bile duct where flukes are not resident (Sripa and Kaewkes 2000).

Several studies report that *O. viverrini* infection is a relative risk to CCA via chronic inflammation (Yongvanit et al. 2012; Pinlaor et al. 2004a; Satarug et al. 1998). Host immune responses can mediate further consequent pathogenesis such as releasing of growth factors, proteolytic enzymes, and fibrogenic cytokines in response to local mechanical and immune irritation to parasite infection, resulting in a persistent inflammatory condition which contributes to the disease and carcinogenesis (Prakobwong et al. 2010; Prakobwong et al. 2009; Sripa et al. 2007). This immunologic reaction also leads to excessive production of reactive oxygen species (ROS) and reactive nitrogen species by inflammatory cells and epithelial bile duct lead to induce oxidative and nitrative DNA damage which may be involved in the initiation and promotion step of experimental cholangiocarcinogenesis (Prakobwong et al. 2010; Pinlaor et al. 2004a). The regulation of inflammatory mediators, particularly cytokines and oxidative stress-related pathways, is believed to mediate antioxidant/pro-oxidant mechanisms (Haddad and Harb 2005; Strober and James 1986). We have also demonstrated a model of inflammation-mediated carcinogenesis via nitric oxide (NO)-mediated oxidative and nitrative DNA damage in hamsters infected with *O. viverrini* (Pinlaor et al. 2004b). Subsequently, we have shown that hamsters infected with *O. viverrini* more than one time (re-infection) induced greater expression of apoptosis-related genes (Boonmars et al. 2007) and accumulation of DNA damage than a single dose through chronic inflammation (Pinlaor et al. 2004a). More recently, we also reported that urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), an oxidative DNA damage marker, level was reported that urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), an oxidative DNA damage marker, level was observed in the order of CCA patients > healthy subjects and urinary 8-oxodG level was observed in the order of CCA patients > healthy subjects and urinary 8-oxodG level was more than one time (re-infection) induced greater expression of apoptosis-related genes (Boonmars et al. 2007) and accumulation of DNA damage than a single dose through chronic inflammation (Pinlaor et al. 2004a). More recently, we also reported that urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), an oxidative DNA damage marker, level was observed in the order of CCA patients > *O. viverrini*-infected patients > healthy subjects and urinary 8-oxodG level was significantly correlated with its levels in leukocytes (Thanan et al. 2008). In addition, we detected in opisthorchiasis subject the etheno-DNA base adducts, 1,N6-etheno-2′-deoxyadenosine (εdA) and 3,N8-etheno-2′-deoxyctydine (εdC) in white blood cell DNA and also as excretion products in urine, probably as a consequence of DNA repair (Dechakhampu et al. 2010; Dechakhampu et al. 2008). These findings demonstrated that inflammation-mediated DNA damage does not occur only at the site of inflammation but also in peripheral blood leukocytes (PBL) of *O. viverrini* infection. However, the mechanism of cellular immune and oxidative stress response to *O. viverrini* infection leading to CCA genesis remains unclear. Therefore, we hypothesized that *O. viverrini* antigen would induce cellular immune response via inflammatory/anti-inflammatory cytokines resulting in induction of oxidant related gene and adaptive anti-oxidant defense mechanism.

To clarify this hypothesis, the PBL taken from *O. viverrini*-infected patients was treated with *O. viverrini* antigen *in vitro*. Expression of genes involved in inflammation including oxidant-generating (toll-like receptor 2 (TLR-2), nuclear factor-kappa B (NF-κB), and cyclooxygenase 2 (COX-2)), anti-oxidant-generating (manganese superoxide dismutase 2 (SOD-2) and catalase (CAT)), inflammatory cytokine (interleukin (IL)-1β) and anti-inflammatory cytokine (IL-10) against *O. viverrini* antigen in PBL was examined in *O. viverrini*-infected patients compared with age- and sex-matched healthy individuals using quantitative RT-PCR analysis *in vitro* co-culture. In addition, lipopolysaccharide (LPS)-treated cells were used as a positive control.

**Materials 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 were divided into two pair-matched groups (matched for age and sex), namely, *O. viverrini*-infected patient and healthy individual groups. All participants volunteered for this study gave informed consent. Healthy individuals (*n* = 4; three males and one female; age, 27–48 years) were defined as persons who are living in Khon Kaen Province and never had a history of *O. viverrini* infection as indicated by negative results for *O. viverrini* antibody and the absence of parasite egg in feces evaluated by modified formalin ether concentration technique. *O. viverrini*-infected patients (*n* = 6; five males and one female; age, 35–50 years) who lived in an endemic area in Khon Kaen Province, Thailand and were positive for only *O. viverrini*-egg count and its antibody. Obese, diabetic patients, and patients with chronic inflammation diseases such as hepatitis virus and tuberculosis infections were excluded from the study. In addition, to exclude urinary tract infection and/or other related inflammatory disease, we examined the morning urine of all subjects by using urinary test kits. Positive urinary nitrate/nitrite in urine was also exclusion criteria.

**Preparation of metacercariae and adult *O. viverrini***

*O. viverrini* metacercariae were obtained from naturally infected cyprinid fish and were digested by artificial pepsin digestion as previously described (Pinlaor et al. 2004b). Cyprinid fish was purchased from the endemic area in Khon Kaen Province. Fish was chopped into small pieces and minced using an electrical blender with freshly prepared...
solution containing 0.25% artificial pepsin and incubated at 37°C in a shaking water bath for 1 h. After digestion, the digested content was filtered and washed several times with normal saline. Metacercariae of *O. viverrini* were collected and identified under a dissecting microscope. Fifty actively viable cysts were fed to Syrian golden hamsters. Adult worms were collected after 1–4 months post-infection. The Animal Ethics Committee of Khon Kaen University, Khon Kaen, Thailand (AEKKU0012/48) approved this study.

Preparation of *O. viverrini* antigen

*O. viverrini* adult were used for crude extract preparation (Pinlaor et al. 2005). After hamsters were infected with *O. viverrini* for 1–4 months, animals were killed, and adult worms were obtained from livers and bile ducts. Complete and fresh parasites were washed several times in a cold normal saline solution containing penicillin (200 U/ml) and streptomycin (200 U/ml). To remove any debris and residual blood, parasites were washed twice with sterile phosphate-buffered saline (PBS) containing penicillin (100 U/ml) and streptomycin (100 U/ml). Subsequently, they were crushed and ground on ice in PBS containing protease inhibitors (Protease inhibitor cocktail, BioRad, CA, USA). The suspension was sonicated, centrifuged at 10,000×g for 30 min at 4°C, and the supernatant was filtered with 0.22-μm filter with sterile technique. Protein concentration of *O. viverrini* antigen was determined by Coomassie Plus™ Protein Assay Reagent (Rockford, IL, USA) following the manufacturer’s protocol. The solution was aliquot with sterile technique and stored at −80°C until used.

White blood cell isolation and *in vitro* co-culture

To clarify the inflammation-mediated pathogenesis, we isolated all population of PBL and treated with parasite antigen. Twelve-hour fasting peripheral blood was obtained by sterilized venipuncture, collected in tubes (10 ml per tube) with heparin, centrifuged at 1,500 rpm at 25°C for 15 min, then the buffy coat and plasma were isolated with sterile technique. To obtain PBL pellets and to remove other contaminated cells, the buffy coat was treated with red blood cell lysis buffer and washed twice with sterile RPMI-1640 medium supplemented with 1-glutamine without Phenol Red (PAA Laboratories, Austria), 2% fetal calf serum gold (FCSg; Laboratories, Austria), and 100 units/ml penicillin and 100 μg/ml streptomycin (Invitrogen, CA, USA). Then, the pellet was washed with RPMI-1640 medium containing 20% FCSg and 1% penicillin/streptomycin. PBL pellet was re-suspended in RPMI-1640 medium containing with 20% FCSg and 1% penicillin/streptomycin. Cell was cultured overnight in a 24-well plate (Corning Incorporated, NY, USA) with 10⁶ cells/ml/well for at 37°C with 5% CO₂ incubator for 24 h. The primary PBL culture was treated with 100 μg/ml/well of *O. viverrini* worm antigen or 1 μg/ml/well of LPS (Sigma, MO, USA) for 3, 6, 12, 24, and 72 h. PBS-treated cell or untreated cell was used as a negative control and LPS-treated cell was used as a positive control. After stimulation, cells were collected and washed twice with cold PBS. Cell pellets were stored at −80°C until gene expression analysis.

Measurement of mRNA expression by quantitative RT-PCR

Total RNA extraction was isolated from PBL pellets by Trizol® reagent (Invitrogen, CA, USA) following the manufacturer’s protocol. A reverse transcription reaction consisted of 0.2 μg total RNA and random hexamer (2.5 μM), which was heated at 70°C for 10 min. The reaction mixture included the first-stranded cDNA synthesis buffer (75 mM KCl; 50 mM Tris-Cl, pH 8.3; 3 mM MgCl₂), 10 mM DTT, 0.5 mM each dNTP and 200 units reverse transcriptase (Promega, WI, USA). Reverse transcription was carried out using a DNA thermocycler (GeneAmp PCR system 2400, Applied Biosystems, CA, USA) at 25°C for 10 min, 37°C for 1 h, and 95°C for 5 min.

Quantitative PCR was performed using TaqMan® Gene Expression assay kits and the ABI 7500 real-time PCR system (Applied Biosystems, CA, USA). TaqMan® PCR primers and probes for TLR-2 (Hs00152932_m1), COX-2 (Hs00153133_m1), NF-κB (Hs00231653_m1), SOD-2 (Hs00167309_m1), CAT (Hs00156308_m1), IL-1β (Hs99999029_m1), IL-10 (Hs0061622_m1), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 4326317E) were used in this experiment (Applied Biosystems, CA, USA). The reporter dyes of all targeted TaqMan® probes in this experiment were labeled with FAM dye whereas GAPDH probe was labeled with VIC dye. PCR reaction was performed using TaqMan® Gene Expression Master Mix (Applied Biosystems, CA, USA) following the manufacturer’s protocol with duplicated sets. The data cycle threshold (ΔCt) method was performed to determine relative concentration, using GAPDH as the normalizing value. The mRNA expression level of targeted genes compared with the housekeeping gene was calculated as 2−ΔCt. A relative mRNA expression level of targeted genes was calculated from the mRNA expression level in treated PBL (*O. viverrini* antigen or LPS) normalized with the mRNA expression level of control PBL (2−ΔCt experiment/2−ΔCt control).

Statistical analysis

In order to normalize the normal distribution of the data, a Log transformation was performed before statistical analysis. Student’s t-test was used to determine the significance in a normal distribution of independent data between
O. viverrini-infected patient and healthy individual groups. Pearson’s correlation coefficient was used to analyze correlations for parametric data. Statistical analyses were performed by SPSS 16.0 for Windows software. P values of less than 0.05 were considered statistically significant.

Results

*O. viverrini* antigen mediates mRNA expression of inflammatory/anti-inflammatory cytokines

To clarify whether *O. viverrini* antigen induces cytokines associated with inflammation-related genes, we examined pro-inflammatory (IL-1β) and anti-inflammatory (IL-10) cytokine genes in PBL of *O. viverrini*-infected patients and healthy individuals. According to the time profile experiment at 6, 12, 24, and 72 h, both the pro-inflammatory and anti-inflammatory cytokines were detected as the upregulated genes in PBL from *O. viverrini*-infected patient and healthy individual groups after being induced by *O. viverrini* antigen and LPS and had the highest expression level at 6 and 12 h, respectively. IL-1β and IL-10 gene expression levels were not significantly different between PBL from *O. viverrini*-infected patients and healthy individual groups as shown in Fig. 1. *O. viverrini* antigen and LPS-treated PBL showed the same trend of induction.

*O. viverrini* antigen induces the expression of oxidant-generating genes

Since oxidant generates gene expression through TLR-dependent pathway and those that is involved in oxidative stress, we examined *O. viverrini* antigen-driven TLR-2, NF-κB, and COX-2 mRNA expression in PBL of *O. viverrini*-infected patient and healthy individual groups. According to the time profile experiment, TLR-2, NF-κB, and COX-2 gene expressions were detected as upregulated genes after the induction of *O. viverrini* antigen and LPS when compared with untreated and have the highest expression level at 6, 12, and 6 h, respectively. Interestingly, the expression levels of TLR-2 and COX-2 in *O. viverrini* antigen-treated PBL in *O. viverrini*-infected patient group were significantly higher than in healthy individual group. Moreover, the expression level of NF-κB expression level in *O. viverrini* antigen-treated PBL from *O. viverrini*-infected group was higher than healthy individual group as shown in Fig. 2. *O. viverrini* antigen- and LPS-treated PBL showed the same trend of induction.

![Fig. 1](https://example.com/figure1.png) The relative mRNA expression of pro-inflammatory (IL-1β) and anti-inflammatory (IL-10) genes in PBL treated with *O. viverrini* antigen and LPS of *O. viverrini*-infected patients (OIP; gray bar) and in healthy individuals (HI; white bar). a The relative expression of IL-1β in *O. viverrini* antigen and LPS-treated PBL from OIP and HI groups at 6 h. b The relative expression of IL-10 in *O. viverrini* antigen and LPS-treated PBL from OIP and HI groups at 12 h. The data are shown as mean±SE of four healthy and six *O. viverrini*-infected subjects (Log scale). Transformed Log scale data were used for the statistical analysis between HI and OIP group by using Student’s *t* test. The relative mRNA expression levels were adjusted by each control experiment (untreated) for individual samples.

*O. viverrini* antigen upregulates expression of SOD-2 but downregulates CAT anti-oxidant genes

To clarify whether *O. viverrini* antigen may induce antioxidant gene association with inflammation-related genes expression, we examined SOD-2 and CAT genes in PBL of *O. viverrini*-infected patient and healthy individual groups. The time profile alteration of the expression levels of SOD-2 and CAT genes had the highest expression at 12 h.
SOD-2 was detected as upregulated gene whereas CAT was detected as downregulated gene in PBL treated with *O. viverrini* antigen and LPS from both *O. viverrini*-infected patient and healthy individual groups. Interestingly, relative SOD-2 expression level in *O. viverrini*-antigen-induced PBL from *O. viverrini*-infected patient group were significantly higher than healthy individual group, whereas relative CAT gene expression levels showed similar expressed level between the *O. viverrini*-antigen-treated PBL from *O. viverrini*-infected patient and healthy individual groups as shown in Fig. 3. *O. viverrini* antigen and LPS-treated PBL showed the same trend of induction.

*O. viverrini* antigen induces ratio of oxidant/anti-oxidant genes

The ratio of TLR-2, NF-κB, and COX-2 by CAT expression levels are shown in Fig. 4. *O. viverrini* antigen significantly increased the ratio of TLR-2/CAT at 6-h post-treatment in *O. viverrini*-infected patient group, which was higher than that in the healthy individual group. A similar result was obtained for the LPS treatment. *O. viverrini* antigen and LPS also increased the ratio of NF-κB/CAT and COX-2/CAT expression levels in *O. viverrini*-infected patient group higher than that in the healthy individual group, but this was not statistically significantly different probably due to a low sample size.

Correlations of inflammation-related genes expression level

A correlation of inflammation-related gene expression level induced by *O. viverrini* antigen was performed by linear regression analysis, and the results are shown in Table 1. Pearson’s correlation test of the data in Log scale (Log $2^{-\Delta \Delta C_{t}}$) obtained from all subjects (healthy and *O. viverrini* infection subjects) and experimental tests (untreated control and treated with *O. viverrini* antigen or LPS) were included. The expression of oxidant-generating genes (TLR-2, NF-κB, and COX-2) and anti-oxidant gene (SOD-2) levels were positively significantly correlated with pro-inflammatory (IL-1β) and anti-inflammatory (IL-10) cytokines. In contrast, anti-oxidant gene (CAT) was positively significantly correlated with only anti-inflammatory cytokine (IL-10). The positive correlations were shown among oxidant-generating genes expression (TLR-2, NF-κB, and COX-2) and the anti-
oxidant enzyme (SOD-2). In addition, CAT mRNA expression level was positively significantly correlated with SOD-2 gene expression in which both gene products function as antioxidant enzymes.

Discussion

In order to test whether those who have been exposed to *O. viverrini* or the healthy control have different inflammatory response to parasite antigen, PBL isolated from *O. viverrini*-infected and uninfected healthy control was treated with parasite antigen. This study reports for the first time an association between transcript expression of cytokines and inflammatory related genes including pro- and anti-inflammatory cytokines, oxidant-generating, and antioxidant genes to *O. viverrini* infection. Our results showed that *O. viverrini* antigen increased IL-1β, IL-10, TLR-2, NF-κB, COX-2, and SOD-2 gene expression levels but do not alter the anti-oxidant gene expression, such as CAT in PBL obtained from *O. viverrini*-infected patients and in healthy individuals. Moreover, the alteration of these genes was rapid and highly responsive in PBL isolated from *O. viverrini*-infected patients at 6 h. The expression level ratio of oxidant-generating/CAT expressions gene levels were higher in infected patients than in healthy individuals. In addition, expression of oxidant-generated genes was found to be positively correlated with the expression of IL-1β and IL-10 cytokine genes, but the ratio of IL-1β/IL-10 was not different between *O. viverrini*-infected and uninfected groups. These results indicate that re-exposure to *O. viverrini* rapidly induces regulation of inflammatory mediators and SOD-2 while CAT expression remained unchangeable. Therefore, this may lead to imbalanced expression of adaptive response genes in cellular antioxidant system.

Expression of oxidant-generating genes (TLR-2, NF-κB, and COX-2) leads to oxidative stress (Dai et al. 2007; Al-Ashy et al. 2006; Li and Verma 2002; Ginn-Pease and Whisler 1998), and we found that these genes are responsible to *O. viverrini* antigen in *O. viverrini*-infected patients being at a higher level than in healthy individuals (Fig.1). This observation can be explained by the expression of their mediators, IL-1β and IL-10, to *O. viverrini* antigen which showed a positive correlation with these oxidant-generating genes (Table 1). Expression of TLR-2 responsible to *O. viverrini* antigen significantly increased at the same time point for PBL of both groups, suggesting that *O. viverrini* antigen may increase activation of an inflammation-related pathway faster than 6 h. The higher responses to *O. viverrini* antigen in infected patients than in healthy individuals suggest that memory T cell responses are faster and more robust than those of their naive counterparts (Dutton et al. 1998).

Expression of these molecules may involve in the inflammatory-mediated pathogenesis and carcinogenesis (Pinlaor et al. 2005). IL-1β is known to induce the synthesis of the enzyme COX-2 through the activation and translocation of p65 subunit of NF-κB (Al-Ashy et al. 2006). IL-1β and IL-10 cytokines are regulated by NF-κB (Dai et al. 2007). Alternatively, *O. viverrini* antigen may induce an inflammatory response through a TLR-2-mediated pathway leading to NF-κB-mediated expression of iNOS and COX-2 (Karin and Greten 2005). These results are in agreement with results in a mouse macrophage cell line which showed an increase of TLR-2, iNOS, and COX-2 at the protein level.
to *O. viverrini* antigen (Pinlaor et al. 2005). TLR signals stimulate not only pro-inflammatory cytokines such as IL-1β but also anti-inflammatory cytokines such as IL-10 and IL-6 (Ozato et al. 2002). Thus, we could not exclude IL-6 cytokine which is considered to be associated with cholangiocarcinogenesis (Sripa et al. 2009). In our model as shown in Table 1, infection with *O. viverrini* may enhance both IL-1β and IL-10 expression, and additionally activate TLR-2 leading to NF-κB-mediated pathway (Dai et al. 2007; Al-Ashy et al. 2006; Li and Verma 2002; Ginn-Pease and Whisler 1998).

Decreasing potential of antioxidant system is well recognized due to oxidative stress. Several antioxidant genes including CAT showed downregulation in animal model of *O. viverrini* antigen. TLR signals induce the imbalance of oxidant/anti-oxidant ratio. Ratio of mRNA expression level of oxidant-generating genes (TLR-2, NF-κB, and COX-2) was divided by anti-oxidant (CAT) gene level and compared with *O. viverrini*-infected (OIP) and healthy (HI) subjects. A Student *t* test was used to test for significant differences.

Table 1 Pearson’s correlation among gene expression levels (Log 2 ΔCT)

<table>
<thead>
<tr>
<th></th>
<th>IL-1β</th>
<th>IL-10</th>
<th>TLR-2</th>
<th>NF-κB</th>
<th>COX-2</th>
<th>SOD-2</th>
<th>CAT</th>
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<tbody>
<tr>
<td>IL-1β</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>IL-10</td>
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<td>1</td>
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</tr>
<tr>
<td>TLR-2</td>
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<td>0.561**</td>
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<tr>
<td>NF-κB</td>
<td>0.381**</td>
<td>0.460**</td>
<td>0.549**</td>
<td>1</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>COX-2</td>
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<td>0.475**</td>
<td>0.450**</td>
<td>0.772**</td>
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<td></td>
</tr>
<tr>
<td>SOD-2</td>
<td>0.485**</td>
<td>0.609**</td>
<td>0.494**</td>
<td>0.702**</td>
<td>0.623**</td>
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<td></td>
</tr>
<tr>
<td>CAT</td>
<td>0.123</td>
<td>0.219*</td>
<td>0.368**</td>
<td>0.290**</td>
<td>0.100</td>
<td>0.449**</td>
<td>1</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01
opisthorchiasis-associated cholangiocarcinoma detected by a kinetic analysis of cDNA microarray technique (Wu et al. 2011). In agreement with other liver fluke, Fasciola hepatica, antioxidant potential was decreased in rat livers after post-infection (Kolodziejczyk et al. 2005). Here, we demonstrated that the expression levels of SOD-2 and CAT genes had a similar trend in healthy individuals and O. viverrini-infected groups. SOD-2 gene expression increased when treated in PBL with O. viverrini antigen whereas CAT seems to be suppressed in both groups. Our result showed that SOD-2 was positively correlated with oxidant-generating genes, suggesting its expression is the first line to catalyze ROS generation (Feng et al. 1995). On the other hand, CAT expression was suppressed in both groups but more significantly lowered in the infected group, suggesting an increased oxidative stress by a decrease in anti-oxidant of CAT during the inflammatory response due to the detoxification function of hydrogen peroxide (H$_2$O$_2$) (Lupertz et al. 2008; Canonico et al. 1977). An adaptive imbalance of anti-oxidant enzymes to genotoxic stress can be the pro-carcinogenic effect which has been proposed by Hofseth et al. (2003). Our result showed that IL-10 was positively correlated with CAT expression, suggesting that the expression of IL-10 is also involved in the reduction of oxidative stress.

All population of white blood cell counts in uninfected and O. viverrini-infected groups were in the normal range. It was previously demonstrated that the eosinophil counts between these two groups also showed no significant change (Pungpak et al. 1994); here, we, therefore, include all populations of leukocytes, including mononuclear cells, neutrophils, and eosinophils; infection by O. viverrini induces a cellular immune response by the induction of oxidant-generating genes but downregulation of anti-oxidant gene (CAT), which may be involved in oxidative stress. A previous human study in an endemic area showed that host cellular immune responses to chronic infection increased endogenous NO production which contributed to form a proinflammation system, which may be one of the key-driven forces for cholangiocarcinogenesis. The present study provides insight into the selection of appropriate chemopreventive agents against opisthorchiasis in endemic areas for the reduction of the disease and CCA, and in the particular area where re-infection occurs more frequently.

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References


Haddad JJ, Harb HL (2005) L-gamma-glutamyl-L-cysteinyl-glycine (glutathione; GSH) and GSH-related enzymes in the regulation of pro- and anti-inflammatory cytokines: a signaling transcriptional scenario for redox(y) immunologic sensor(s)? Mol Immunol 42(9):987–1014