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• LIVER CANCER •

Oxidative DNA damage in peripheral leukocytes and its association with expression and polymorphisms of $hOGG_1$: A study of adolescents in a high risk region for hepatocellular carcinoma in China

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Abstract

AIM: To study the oxidative DNA damage to adolescents of hepatocellular carcinoma (HCC) families in Guangxi Zhuang Autonomous Region, China.

METHODS: Peripheral leukocytes' DNA 7, 8-dihydro-8-oxoguanine (8-oxoG) and repair enzyme $hOGG_1$ were quantified by flow-cytometry. $hOGG_1$-Cys326Ser single nucleotide polymorphism (SNP) was distinguished by polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) assay.

RESULTS: There was a positive correlation between 8-oxoG and repair enzyme $hOGG_1$ expression ($P<0.001$). HCC children ($n=21$) in Fusui county had a higher level of $hOGG_1$ ($P<0.01$) and a lower level of 8-oxoG ($P<0.05$) than the controls ($n=63$) in Nanning city. Children in Nanning exposed to passive-smoking had a higher $hOGG_1$ expression ($P<0.05$) than the non-exposers. 8-oxoG and $hOGG_1$ were negatively correlated with body mass index, while $hOGG_1$ was positively correlated with age. There was a peak of 8-oxoG level nearby the 12 year point. Individuals with the $hOGG_1$ 326Ser allele had a significantly marginal higher concentration of leukocyte 8-oxoG level than $hOGG_1$ 326Cys allele.

CONCLUSION: This is the first report using flow-cytometry to simultaneously quantify both the DNA oxidative damage and its repairing enzyme hOGG1. The results provide new insights towards a better understanding of the mechanisms of oxidative stress in a population highly susceptible to hepatocarcinogenesis.

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INTRODUCTION

Reactive oxygen species (ROS) possess a high reactivity of a variety of biological molecules, among which, DNA is one of the most important targets[2]. Oxidative DNA damage, caused by either endogenous or exogenous source of ROS, has been linked to aging, chronic degenerative diseases, inflammatory diseases and cancers[3-6]. Among various types of DNA base modifications induced by ROS attack, 7,8-dihydro-8-oxoguanine (8-oxoG) has been the most widely studied and is considered as a key biomarker of oxidative DNA damage[7]. Leaving unrepaired, 8-oxoG is highly mutagenic because of its propensity to mispair with adenine during DNA replication, ultimately yielding GC to TA transversion[8].

To minimize 8-oxoG accumulation within genomes, this lesion is subjected to DNA repair primarily through the base excision repair pathway[9]. A key component of this pathway in eukaryotes is OGG_1, a DNA glycosylase/β-lyase that recognizes 8-oxoG opposite cytosine[10]. Inactivation of the OGG_1 gene generates a mutator phenotype characterized by GC-TA transversions in yeast[10]. Analysis of the human OGG_1 gene ($hOGG_1$) and its transcripts in normal and tumoral tissues has revealed alternative splicing, polymorphisms and somatic mutations[11]. The repair effectiveness of OGG_1 may be modulated by gene polymorphisms. A Cys326Ser substitution in exon 7 has been the most extensively studied. The Cys326 isoform is postulated to exhibit reduced 8-oxoG repair activity[12], increase susceptibility to squamous cell carcinoma of lung cancer[13,14], otolaryngeal cancer[15] and esophageal cancer[16], nevertheless, controversy still remains[17-21].

Dietary aflatoxin exposure[22,23] and hepatitis infection[24,25] are two well known risk factors in liver carcinogenesis, which involves ROS generation and oxidative DNA damage. A synergistic effect of aflatoxin B_1 (AFB_1) and hepatitis virus B (HBV) may be involved in the hepatocellular carcinoma (HCC) formation, and may be responsible for the predominance of one hotspot GC→TA transversion in the p53 gene of affected individuals[22,23].

HCC is the third most common cause of cancer death in China[26], and the main killer in a south-western province, Guangxi Zhuang Autonomous Region[27]. The age-standardized mortality of HCC for males and females in this province was 32.5/100 000 and 8.5/100 000, respectively[1], accounting for 50 % and 25 % of all the cancer deaths in this region in men and women, respectively[1]. Thus far, dietary AFB_1 exposure[28] and HBV infection[1] are the well documented risk factors

for the extraordinarily high prevalence of HCC in this area. Our earlier data[29,30] together with that of Stern *et al*[31] have highlighted a frequency of 36-73 % of p53-249 codon mutation in HCCs in this region, which is consistent with the notion that, the p53-249 hot-spot mutation is a fingerprint of AFB_1 contamination, and possibly synergistic with HBV infection in hepatocarcinogenesis[22].

In the present study, on the assumption that environmental carcinogens may impose oxidative stress on the residents living in Guangxi Zhuang Autonomous Region, we examined the level of 8-oxoG and $hOGG_1$ expression in leukocytes of a random sample of adolescents aged 4-18 in an area of Guangatxi exposed to a high level of aflatoxin and high risk of HCC, using a newly developed flow cytometry method. Furthermore, we examined the relationship between DNA damage and genetic polymorphisms of oxidative damage repair gene $hOGG_1$.

MATERIALS AND METHODS

Study subjects

This collaborative study by the Guangxi University and Fusui Cancer Institute was part of a community-based health survey in the Nanning region of Guangxi Zhuang Autonomous Region, conducted during April to June 2001. Based on the local cancer registry from 1974 to 1999, the HCC incidence rate in this region ranged from 32 to 97 per 100 000 for males and 4.27 to 17.32 per 100 000 for females, respectively. The aims of the study were explained in detail prior to the survey. From a total of 472 informed residents, 162 adults and 123 adolescents (60.4 % response rate) participated in on a voluntary basis.

After written consents were obtained from all the individuals or children' s parent/guidance. Ten-milliliter venous blood was collected with heparin as anticoagulant. Buffy-coat and plasma separated soon after collection, and kept in liquid-nitrogen during transportation, and stored at -80 ℃ till analysis. Body weight, height, age, gender, occupation, alcohol and smoking habit and family history of hepatitis infections were also recorded using a structured questionnaire approved by the Guangxi Medical University. Plasma of all subjects was screened to differentiate HBV, HCV, HDV, HEV and HGV infections. 25.6 % (73/285) subjects were positive of HV infection (63/HBV(+), 1/HBV&HCV(+), 1/HCV(+), 4/HBV&HDV(+), 2/HEV(+), 2/HBV&HGV(+)). These cases were excluded, in order to rule out the possibility of influence from hepatitis viruse infection[24,25]. Furthermore, subjects with known exposure to known environmental or occupational hazards (such as cigarette smoking, alcohol or pesticides), as well as those who were currently under medication or known to have a chronic illness were also excluded. Fifty-six boys and 28 of girls aged from 4 to 18 (mean±SD, 11.45±3.0 years) born and grown up in this region met the above mentioned criteria, and were selected for this investigation.

Determination of DNA 8-oxoguanine and $hOGG_1$ in leukocytes

Prior to the investigation, extensive experiments were conducted to optimize the antibody/probe titers and the amount of cells to be used for flow cytometry. It was found that reproducible data could be achieved with 40 μl-buffy coat (around $0.8\text{-}1.1\times10^6$ WBC). The buffy-coat was first transferred from -80 ℃ to 4 ℃, gently thawed for 4 h, and then shifted to room temperature till completely thawed. Forty micro liters buffy-coat were counted for leukocytes using a hemocytometer, and another 40 μl buffy-coat was transferred into an Eppendorf tube containing 1 ml of PBS with 1 % paraformaldehyde, stood on ice for 30 min. After washed twice with PBS, Cells were then fixed in ice-cool 70 % ethanol and kept at -20 ℃ till staining.

8-oxoG was stained by a Biotrin OxyDNA Assay Kit (Fluorescein isothiocyanate, FITC-conjugated probe, Biotrin Int. Ltd., Dublin, Ireland.) according to the manufacturer' s protocol with some minor modifications[32,33]. For $hOGG_1$ staining, first antibody (1st Ab, goat-anti-$hOGG_1$) was obtained from Santa Cruz Biotech, Inc (California, U.S.A.) (goat polyclonal antibody, against a peptide mapping at the amino terminus of OGG_1 of human origin, reacts with all OGG_1 splice variants of human origin). The second antibody (2nd Ab, R-phycoerythrin, PE-conjugated rabbit anti-goat IgG) was purchased from Sigma-Aldrich Inc., (St. Louis, MO, USA). Before staining, the cells were treated by Biotrin Blocking Solution at 37 ℃ for 60 min. After washed with Biotrin Washing Solution, the cells were first incubated with 1st Ab (goat-anti-$hOGG_1$, 1:100 in 3 % FBS/PBS), followed by a mixture of Biotrin 8-oxoG probe (FITC-conjugate, 1:10 dilution) and 2nd Ab (rabbit-anti-goat, PE conjugate, 1:400 dilution) in the Biotrin Washing Solution at 37 ℃ in the dark for 60 min per step. The cells were then re-suspended and quantified by a flow cytometer (Coulter Epics Elite Flow Cytometer, Coulter Corporation, Miami, USA), at 488 nm excitation and 525 nm (FITC), 578 nm (PE) emission, respectively. Blood from a healthy adult volunteer was included and served as an internal control. Data were analyzed using the WinMDI2.8 software (http: //facs.scripps.edu/software.html). 8-oxoG and $hOGG_1$ levels were determined as percentage of positive-staining-cells (oxoGP, $hOGG_1P$) and mean of relative fluorescence intensity (RFI for oxoGI, $hOGG_1I$) of 10 000 cells counted by the flow cytometer[34]. To eliminate possible batch-to-batch variations, all the samples were analyzed at the same batch by the same cytometer.

Genotyping of $hOGG_1$ Cys326Ser single nucleotide polymorphism (SNP)

PCR-SSCP analysis of $hOGG_1$ Cys326Ser SNP was modified from a technique described by Kohno *et al*[12]. DNA extracted from leukocytes using phenol-chloroform method was amplified by PCR. The primers used were 5' -actgt-cacta-gtctc-accag-3' (forward) and 5' -tgaat-tcgga-aggtg-cttgg-ggaat-3' (reverse) (Research Biolabs Pte., Ltd., Singapore). PCR polymerase and dNTP were from Finnzymes (ESPOO, Finland). Twenty-μl PCR reaction mixture contained 2 μl 10×PCR-buffer, 1.5 mM $MgCl_2$, 0.1 mM dNTP, 0.5 μM of each primer, 1 U Finnzyme polymerase and -100 ng of sample DNA. PCR reaction on a thermocycler (Biometra TGradient, Göttingen, Germany) began with pre-incubation at 94 ℃ for 5 min, followed by 30 cycles of denaturation at 94 ℃ for 30 s, annealing at 54 ℃ for 30 s, and elongation at 72 ℃ for 30 s. PCR products were diluted with 4 volumes of loading buffer (0.5×TBE, 0.05 % bromophenol blue, 0.05 % xylene cyanol, 20 mM methylmercury hydroxide), heat-denatured at 85 ℃ for 5 min and rapidly cooled on ice before loading. Twenty microliters of each sample were separated on 10 % non-denaturing polyacrylamide gels with or without 5 % glycerol. Electrophoresis was conducted at 200 V constant for 5 h at 4-5 ℃. Silver staining followed Forsberg *et al*[35]. Genotype of each band-pattern was confirmed by a subsequent sequencing in a commercial laboratory (Research Biolabs Pte., Ltd, Singapore) using BigDye™ Terminator kits on a ABI PRISM® 377-96 DNA Sequencer. A blank was inserted into each batch PCR to monitor PCR contamination, and for each sample, at least one independent repeat of PCR-SSCP assay was done.

Statistical analysis

All analyses were performed using the SPSS 10.0 program (Chicago, USA). Two cases were excluded from flow cytometry assays because of inadequate leukocytes. The frequency distributions of 8-oxoGP, 8-oxoGI, $hOGG_1P$ and $hOGG_1I$ skewed to the right while body mass index (BMI)

skewed to the left. In order to obtain acceptable fit to the normal distribution and stabilize the variance, 8-oxoGI, $hOGG_1I$ and BMI were log-transformed while 8-oxoGP and $hOGG_1P$ were transformed by the formula: log ((100+X)/(100-X)). Non-parameter Mann-Whitney test and one-way ANOVA were used to examine the influence of gender and $hOGG_1$ genotype on the four biomarkers, respectively. A partial correlation was calculated to examine the closeness of relationship between continuous variables (biomarkers, age and BMI). Multiple linear regressions were conducted to examine the influence of risk factors on the four biomarkers, respectively. A two-tailed *P* value <0.05 was considered significant.

RESULTS

Levels of 8-oxoG and $hOGG_1$

Figures 1a to 1d show the images of leukocytes stained for 8-oxoG and $hOGG_1$ under laser confocal microscope. The mean levels of the four biomarker parameters in male and female children are summarized in Table 1. The positive-staining cell percentage of 8-oxoG and $hOGG_1$ ranged between 45-99 % and 44-98 %, respectively. It was noted that the majority (>72 %) of cases had over 90 % positive-staining leukocytes for both 8-oxoG and $hOGG_1$. The data suggested that between male and female children, there was no significant difference in both 8-oxoG and $hOGG_1$ expressed either as percentage or fluorescent intensity.

Association between 8-oxoG, $hOGG_1$, age and BMI

Table 2 reveals that there were significant correlations between 8-oxoG level and $hOGG_1$ expression even after adjustment of age and BMI. Furthermore, a positive correlation was seen between age and $hOGG_1$ expression, either measured as percentage or intensity. In contrast, a negative correlation was noted between BMI and 8-oxoGP, and between BMI and $hOGG_1P$. These associations remained significant even when BMI or age was adjusted. These data suggested that age and BMI could independently influence the levels of oxidative damage and $hOGG_1$ gene expression.

Association between biomarkers and $hOGG_1$ Cys326Ser SNP

The median levels and 95 % confidence interval (95 % CI) of the four biomarker parameters of their respective $hOGG_1$ Cys326Ser SNP genotype are presented in Table 3. Children with $hOGG_1$ Cys326Ser heterozygote were found to have a higher level of 8-oxoG than those with Cys/Cys homozygote, although statistical significance was observed only in 8-oxoG intensity. Also, comparison of subgroup Cys/Cys *vs*. (Cys/Ser + Ser/Ser) revealed similar results (data not shown).

Multivariate analysis

To explore possible interactions of risk factors, a linear regression was then conducted for the four biomarkers, respectively (Table 4). Based on the multiple regression analysis against 8 variables, $hOGG_1$ expression level increased with aging, leaner children had a lower level of 8-oxoG, and $hOGG_1$ 326Ser allele had an increasing effect on 8-oxoG level while a reducing effect on $hOGG_1$ expression. However, the 8 factors listed in Table 4 explained only less than 50 % of the entire variations, suggesting that other oxygen radical-forming factors might be involved.

Table 1 Level of biomarkers in male and female children

	8-oxoGP (%)	8-oxoGI (RFI)	$hOGG_1$ (%)	$hOGG_1$ (RFI)	Double staining percentage (%)
Male (*n*=54)	94.55 (89.14, 94.16)[b]	61.74 (58.02, 73.57)	93.29 (88.39, 93.11)	110.50 (100.39, 125.07)	86.76 (81.10, 86.90)
Female (*n*=28)	94.98 (90.03, 95.17)	69.91 (60.28, 78.83)	91.78 (88.31, 92.86)	102.51 (85.39, 116.24)	87.10 (81.14, 87.79)
P[a]	0.469	0.323	0.577	0.225	0.822

A: Non-parameter Mann-Whitney Test, B: Data in cells presented as median (95 % confidence interval).

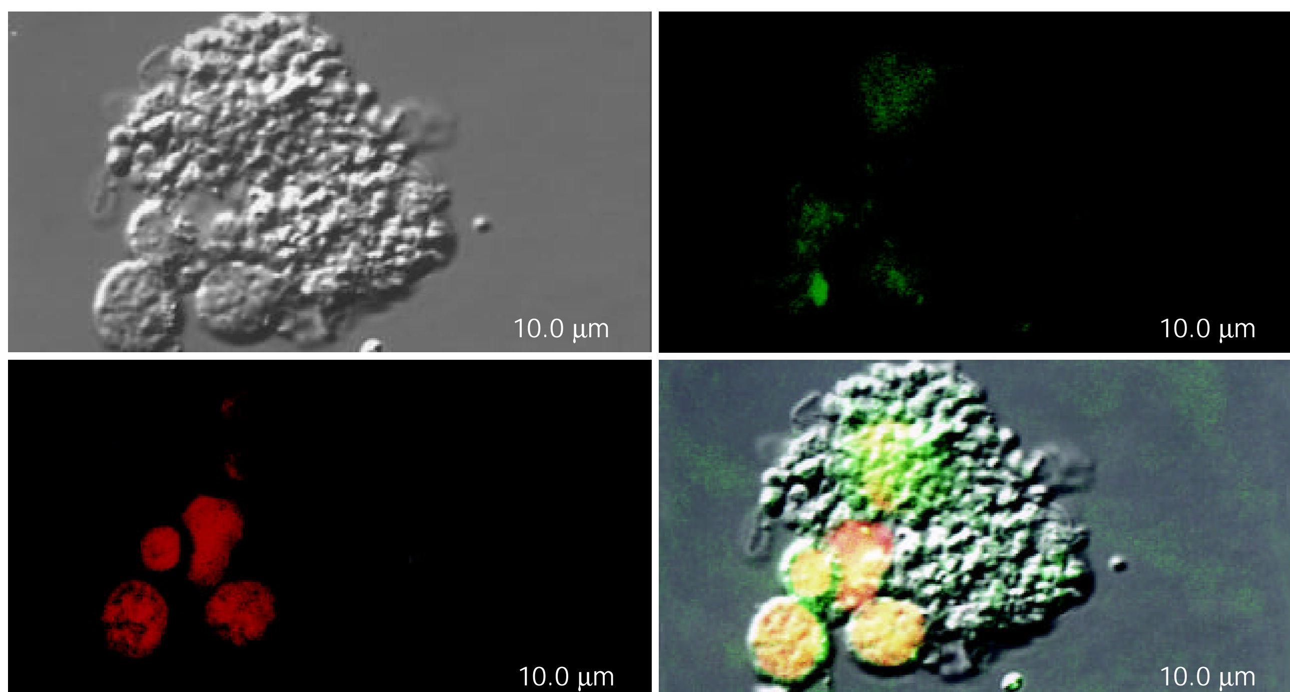

Figure 1 Representative images of leukocytes stained by 8-oxoG-FITC probes (green) and $hOGG_1$-PE complex (red) under laser confocal microscopy. The bottom-right figure demonstrated that some of the leukocytes were simultaneously stained with both FITC and PE complex.

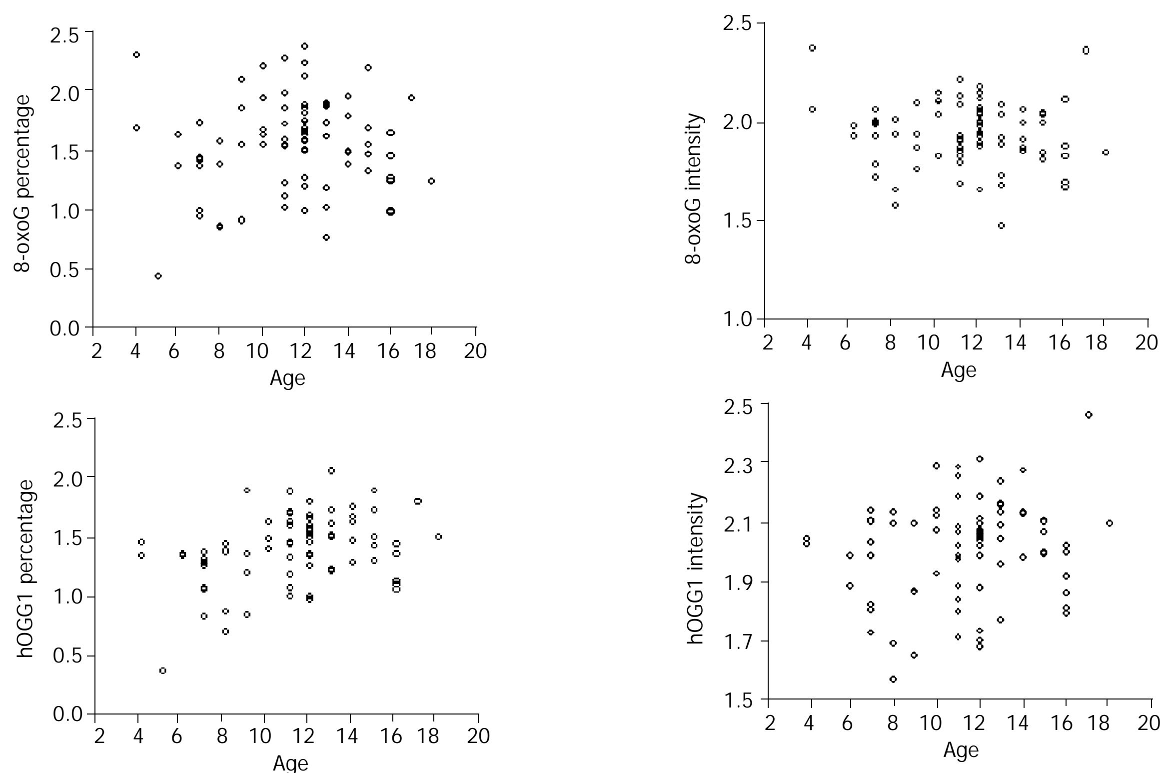

Figure 2 Scatters of age-biomarkers.

Table 2 Partial Correlation between biomarkers, age and BMI

	8-oxoGP (%)	8-oxoGI (RFI)	$hOGG_1P$ (%)	$hOGG_1I$ (RFI)	Double staining percentage (%)
8-oxoGP (%)[a]		0.6987 (0.000)[f]	0.8333 (0.000)[f]	0.5299 (0.000)[f]	0.9553(0.000)[f]
8-oxoGI (RFI)[a]			0.4843 (0.000)[f]	0.5155 (0.000)[f]	0.7075(0.000)[f]
$hOGG_1P$ (%)[a]				0.6727 (0.000)[f]	0.8482(0.000)[f]
$hOGG_1I$ (RFI)[a]					0.6528(0.000)[f]
Age[b]	0.1832 (0.102)	-0. 0326 (0.773)	0.3332 (0.002)[f]	0. 2316 (0.037)[e]	0.1655(0.140)
BMI[b]	-0.3339 (0.002)[f]	-0. 0983 (0.383)	-0. 3111 (0.005)[f]	-0. 0415 (0.713)	-0.2285(0.0040)[f]
Age[c]	0.2414(0.031)[e]	-0.0204 (0.858)	0.3954 (0.000)[f]	0.2390 (0.033)[e]	0.2015(0.073)
BMI[d]	-0.3663 (0.001)[f]	-0.0950 (0.402)	-0.3779 (0.001)[f]	-0.0735 (0.517)	-0.2552(0.022)[e]

A: Adjusted for (gender+age+BMI), B: Adjusted for gender; C:Adjusted for (gender+BMI), D: Adjusted for (gender+age), E: Correlation was significant at the 0.05 level, F: Correlation was significant at the 0.01 level, G: Data presented in cells were correlation coefficient (*P*).

Table 3 Association between biomarkers and $hOGG_1$-326 SNP (Oneway ANOVA)

	8-oxoGP (%)			8-oxoGI (RFI)			$hOGG_1P$ (%)			$hOGG_1I$ (RFI)		
	Median	*P* (LSD)		Median	*P* (LSD)		Median	*P* (LSD)		Median	*P* (LSD)	
	(95 % CI)	Cys/Ser	Ser/Ser	(95 % CI)	Cys/Ser	Ser/Ser	(95 % CI)	Cys/Ser	Ser/Ser	(95 % CI)	Cys/Ser	Ser/Ser
Cys/Cys	94.03	0.075	0.250	58.61	0.023[a]	0.252	93.37	0.633	0.699	116.72	0.714	0.329
(*n*=32)	(85.96, 93.78)			(50.01, 70.31)			(85.58, 93.45)			(94.94, 133.28)		
Cys/Ser	94.73		0.864	71.16		0.573	93.28		0.452	110.90		0.205
(*n*=37)	(91.48, 95.53)			(64.25, 80.99)			(90.26, 93.35)			(97.92, (123.07)		
Ser/Ser	94.98			58.73			91.80			94.93		
(*n*=13)	(88.98, 96.61)			(52.01, 84.67)			(87.18, 93.64)			(73.26, 106.82)		
(F) *P*		(1.751) 0.180			(2.741) 0.071			(0.312) 0.733			(0.821) 0.444	

[a]$P<0.05$.

Table 4 Multiple regression models of predictors on levels of oxidative DNA damage biomarkers

Predictors in the model	Double staining percentage (%)		8-oxoGP		8-oxoGI		hOGG$_1$P		hOGG$_1$I	
	Beta[b]	*P*	Beta	*P*	Beta	*P*	Beta	*P*	Beta	*P*
8-oxoGP							0.463		0.000[c]	
8-oxoGI	0.521	0.000[c]							131.226	0.000[c]
hOGG$_1$P			0.922	0.000[c]						
hOGG$_1$I	0.411	0.000[c]			6.591E-02	0.008[c]				
Age	0.090	0.201	-0.139	0.108	-0.075	0.426	2.532E-02	0.001[c]	3.069	0.018[c]
Gender	-0.027	0.693	-0.081	0.336	-0.106	0.253	0.058	0.474	0.104	0.262
BMI	-0.046	0.496	-0.055	0.511	-1.048E-02	0.050[c]	-0.053	0.516	0.037	0.696
hOGG$_1$ 326SNP	0.056	0.427	0.103	0.029[c]	2.370E-03	0.000[c]	-0.112	0.166	-15.684	0.006[c]
Constant	0.480	0.000	6.969E-02	0.705	1.601	0.000	0.401	0.001	-133.512	0.001
R^2(F)[d]	0.667 (79.227)[e]		0.470 (35.033)[e]		0.377 (15.729)[e]		0.509(40.875)[e]		0.386(16.369)[e]	

A: hOGG$_1$ 326SNP: Cys/Cys=1; Cys/Ser=2; Ser/Ser=3; B: Beta: standardized coefficient; C: Variables entered final equation (backward); D: R square of model (F value of ANOVA); E: ANOVA *P* value <0.01.

DISCUSSION

Population-based researches can provide new clues to etiology of a disease. The unique epidemiology of high mortality rate of HCC in Guangxi Zhuang Autonomous Region, China has provided an ideal population model for the study of oxidative DNA damage and corresponding repair mechanism[1]. Although aflatoxin exposure was not measured in the present study, our earlier reports and others have shown that the dietary intake of aflatoxin in the local residents was high[28,36]. Furthermore, it has been well established that AFB1 causes rapid ROS formation and leads to DNA oxidative damage, which plays a critical role in hepato-carcinogenesis[22]. The subjects of the present study came from a typical HCC high risk community. In order to rule out the possibility of influence from hepatitis virus infection and other potential environmental and occupational factors, such as pesticides, cigarette smoking and alcohol, we have limited the present study to 82 children under the age of 18 with no known liver diseases or other medical history.

Methodology of quantification for oxidative DNA damage

Immunohistochemistry-based approaches have been widely used for the quantification of DNA adducts in various tissues[37], as well as in *in-situ*[38,39] or urinary[40] 8-oxoG quantification. On the other hand, flow cytometry technology allows multi-parameter analyses of heterogeneous cell population, in which immunophenotyping of both surface and cytoplasmic antigens, DNA analysis and functional evaluations are combined. Subsets of cells can be identified and characterized by patterns of maturation antigens and staining intensity[41]. This report is the first study thus far utilizing flow cytometry for simultaneous quantification of both 8-oxoG and a high risk population of HCC as a model. Compared to the immuno-quantification of 8-oxoG using visualized counting of positive-staining cells, the flow cytometry approach we used, has the following advantages. The assessment is more objective compared to other methods as the scoring is not operator dependent, both the number of positive-staining cells and fluorochrome intensity could be quantified simultaneously, the sample required (-1×10^6 cells) is much less, compared to other traditional methods, and high-throughput (it can handle as many as 100 samples per batch, thus eliminating possible batch to batch variations).

Prior to the present investigation, attention was paid during the pilot study to standardize and validate the instrumentation and methodology. Factors such as specificity and performance of the reagents, staining intensity, spectral overlap, and instrument compensation were carefully evaluated[41]. The overall indication is that the flow cytometry determinations of 8-oxoG and hOGG$_1$ are of high reliability. The reproducibility is generally over 90 % with batch-to batch variation of less than 10 %. As for the actual samples from Guangxi, a substantial proportion of leukocytes was found to be positively-stained for both 8-oxoG and hOGG$_1$. These values are much higher compared to normal healthy subjects from Singapore of about 20 % to 40 % (Tao *et al*, unpublished data). Table 2 clearly demonstrates that there were significant correlations between 8-oxoG level and hOGG$_1$ expression, either expressed as percentage or chromophore intensity. Even after confounding factors such as age and BMI were adjusted, there were still significant associations between oxidative damage and hOGG$_1$ expression in leukocytes (Table 2). This finding supports that normal cells have certain defensive mechanism against ubiquitous oxidative DNA damage[9], and hOGG$_1$ works as a housekeeping gene, ubiquitously expressed during cell cycle[42].

Although inter-individual variations of hOGG$_1$ are genetically determined, the present data and several earlier studies suggest that its expression could be influenced by endogenous formation of ROS or environmental carcinogens. Increased 8-oxoG repair activity has been shown to increase in smoker's leukocytes[43] and in lung cells exposed to asbestos[44]. Quantitative assessment of hOGG1 expression in peripheral blood cells can provide information on exposure to environmental carcinogens[45]. The present study has clearly demonstrated that hOGG1 in leukocytes, expressed either in percentage or fluorescence intensity, can indicate the oxidative damage in an HCC high risk population, suggesting that hOGG$_1$ is a useful biomarker for monitoring oxidative stress, in addition to 8oxoG.

Gender and oxidative DNA damage

Based on the epidemiological data obtained earlier in other parts of China as well as in Guangxi Zhuang Autonomous Region, the occurrence of HCC is predominant in men, the ratio of male to female is 4-6:1[1]. However, in the present study, we did not observe any difference in either 8oxoG or hOGG$_1$ level between male and female children (Table 1). According to Loft *et al.*,

adult healthy men aged 40 to 60 excreted 29 % (10-48 %) more 8-oxoG in urine than women[46]. Nevertheless, DNA damage, measured as either percentage of DNA migrated in COMET tail[47] or 8-oxoG excretion in urine[48], was not associated with gender in other two adult populations. In healthy individuals, there was no difference in $hOGG_1$ activity due to gender by means of an 8-oxoG-containing oligonucleotide assay[17]. Since this was a children-based study, further evidences from adult subjects are required to elucidate the role of gender-related factors in hepatocarcinogenesis in this population.

Age, BMI and oxidative DNA damage

An inversed relationship between BMI and urinary excretion of 8-oxoG in adults has been documented[46,49], and was postulated to be due to a higher metabolic rate in lean persons[46]. In the present study, similarly, we observed a negative correlation between BMI and 8-oxoGP, and between BMI and $hOGG_1P$ (Table 2), which was consistent with that in previous reports. There was also a positive correlation between $hOGG_1$ level and age, which was not consistent with that of 8-oxoG (Figure 2, Table 2). This age-dependent increase of $hOGG_1$ in children has never been reported elsewhere. The results indicated that, the age-dependent increase of $hOGG_1$ level can not simply be explained by metabolic rate and BMI.

Using COMET assay to study DNA damage of children in Mexico City, Calderon-Garciduenas[50] showed that there was an age-dependent increase in the percentage of nasal cells with COMET tails >10 microns, implying a dose response relationship between exposure to environment pollutants and increase of age. Drury[51] in a preliminary study of 15 children observed an increase of mean 8-OHdG excretion in urine with postnatal age (r=0.80, P<0.0001). Nevertheless, the author argued that these changes could also be due to changes in the activity of the enzyme responsible for 8-OHdG excision. According to Bogdanov[52], however, neonates of 50-60 days had a higher 8-OHdG excretion in urine (13.39±0.082 ng/mg creatinine, n=150) than children aged 3-9 (4.62±0.091 ng/mg creatinine, n=32). So far no report about hOGG1 expression in children is available. Therefore it is not known whether this age-dependency is attributed to growth-related increase of metabolism or dietary increase (accumulation) of exposure to aflatoxin or other hazards, or both. Further studies with a larger population should be able to provide more insights in this preliminary observation.

$hOGG_1$-Cys326Ser SNP and oxidative DNA damage

DNA repair enzyme OGG1 is a DNA glycosylase/AP lyase that has been hypothesized to play an important role in preventing carcinogenesis by repairing oxidative damage to DNA. Specifically, it can efficiently repair 8-oxoG, a major base lesion produced by ROS and formed by endogenous metabolism or exposure to environmental oxidizing agents or genotoxic compounds[10]. In this study we have analyzed the variants of $hOGG_1$-Cys326Ser in 84 children and the results showed that the frequency of $hOGG_1$-Cys326 allele of 54.8 % was similar to that reported by Sugimura[13] and Takezaki[53] conducted in two other Chinese populations (54.5 % to 60.7 %). In the present investigation, individuals with the $hOGG_1$ 326Ser allele rather than $hOGG_1$ 326Cys allele, had a significantly higher concentration of leukocyte 8-oxoG level (Tables 3 and 4).

Kohn *et al*. first described a reduced repair activity of $hOGG_1$-Cys326 protein in a complementation assay system[12]. Nevertheless, this observation was not supported by another study using cell homogenates-cleavage system[17]. A recent study by Janssen *et al* found that DNA repair activity of OGG1 in human lymphocytes was not dependent on the Ser326Cys variants[17]. Paralleling to the results of functional studies, population studies on $hOGG_1$-326 polymorphism and cancer susceptibility thus far were also not conclusive[17-21]. It is however important to note that the proportion of homozygous Ser-Ser individuals is the highest in Melanesians (74.5 %), and German (57.1 %), lower in Australian Caucasians (40 %), Japanese (27.7 %) and even lower in Chinese (12 %)[13].

Since the 8-oxoG level measured in a tissue at time is an integration of a number of parameters including the level of ROS, tissue redox status, cellular antioxidant defense mechanism and DNA repair system[21], our data suggest that the role of $hOGG_1$ SNP, if any, in modulating 8-oxoG level of individuals, may be diluted by other confounders. On the other hand, it is believed that $hOGG_1$ may not be the only gene associated with oxidative damage. An alternative DNA oxidative damage repair pathway to minimize the effects of 8-oxoG in genomes has also been reported recently[54]. Carefully designed studies considering these confounders however, are obviously needed to verify this observation.

From the data obtained in this study, it is concluded that, oxidative damage is significantly correlated with the DNA damage repair enzyme $hOGG_1$, there are positive associations between oxidative damage, repair enzyme $hOGG_1$ and age, while there are reverse relationships between oxidative damage and body mass indexes, and polymorphism of $hOGG_1$ variant appears to influence 8-oxoG level in peripheral leukocytes, but only at a marginal strength. These findings provide some new insights into a better understanding of the complex etiology and molecular events that could lead to the development of HCC. Further study should take into consideration of both long term environment exposure and genetic susceptibility in a larger population.

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