

RESEARCH ARTICLE

Changes of Oxidative Stress, Glutathione, and Its Dependent Antioxidant Enzyme Activities in Patients with Hepatocellular Carcinoma before and after Tumor Resection

Shao-Bin Cheng^{1,2}, Hsiao-Tien Liu¹, Sin-Yuan Chen³, Ping-Ting Lin^{4,5}, Chia-Yu Lai¹, Yi-Chia Huang^{4,5*}

1 Division of General Surgery, Department of Surgery, Taichung Veterans General Hospital, Taichung, Taiwan, **2** School of Medicine, Chung Shan Medical University, Taichung, Taiwan, **3** Graduate Program in Nutrition, Chung Shan Medical University, Taichung, Taiwan, **4** Department of Nutrition, Chung Shan Medical University, Taichung, Taiwan, **5** Department of Nutrition, Chung Shann Medical University Hospital, Taichung, Taiwan

* yeh@csmu.edu.tw



OPEN ACCESS

Citation: Cheng S-B, Liu H-T, Chen S-Y, Lin P-T, Lai C-Y, Huang Y-C (2017) Changes of Oxidative Stress, Glutathione, and Its Dependent Antioxidant Enzyme Activities in Patients with Hepatocellular Carcinoma before and after Tumor Resection. PLoS ONE 12(1): e0170016. doi:10.1371/journal.pone.0170016

Editor: Sheng-Nan Lu, Chang Gung Memorial Hospital Kaohsiung Branch, TAIWAN

Received: September 1, 2016

Accepted: December 25, 2016

Published: January 12, 2017

Copyright: © 2017 Cheng et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper.

Funding: This study was supported by the Ministry of Science and Technology, Taiwan (MOST 104-2320-B-040-009-MY3) and Taichung Veterans General Hospital (TCVGH-1064602C). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

The changes in and relationship between oxidative stress and the glutathione (GSH) antioxidant system in the plasma and tissues of patients with hepatocellular carcinoma (HCC) before and after tumor resection have not been clearly determined. We investigated the changes in oxidative stress, GSH status and its dependent antioxidant enzyme activities in HCC patients before and after tumor resection, and to determine the association of oxidative stress with GSH and its dependent antioxidant enzyme activities in plasma and tissues. This study employed a cross-sectional design. Forty-four men and 16 women with HCC were recruited. Fasting blood was drawn on the day before the tumor resection and one month after the tumor resection. HCC tissue and adjacent normal liver tissue were obtained at the time of surgical resection. Patients had significantly increased plasma malondialdehyde (MDA) and oxidized-low density lipoprotein levels but decreased GSH and oxidized GSH levels before tumor resection compared with the corresponding post-resection values. GSH and trolox equivalent antioxidant capacity (TEAC) levels and activities of GSH peroxidase were significantly increased while MDA level was significantly lower in HCC tissue when compared with the adjacent normal tissue. The pre-resection plasma MDA level was significantly correlated with pre-resection plasma GSH concentration, and MDA level in HCC and adjacent normal tissues. Pre-resection plasma GSH concentration was significantly correlated with GSH and TEAC level in HCC tissue. HCC patients had increased oxidative stress, decreased GSH, and lower dependent antioxidant capacities before tumor resection. However, hepatocellular tumor had increased GSH and TEAC levels as well as GSH peroxidase activities which might protect itself against increased oxidative stress.

Competing Interests: The authors have declared that no competing interests exist.

Introduction

Hepatocellular carcinoma (HCC) is the most common type of liver cancer and the mortality rate has risen over the past decade. Among risk factors related to HCC, increased oxidative stress and decreased antioxidant capacities have been suggested to be contributors to HCC [1–5]. Although the human body has different antioxidant systems to defend against the generation of free radicals, oxidative stress is elevated when antioxidant capacities are low, which disrupts the balance between pro- and anti-oxidants and further causes the oncogenesis and tumor progression of HCC [6,7].

Glutathione (GSH) is a thiol and tripeptide which is synthesized by glycine, cysteine, and glutamate in the liver and acts as a vital factor in metabolic protective functions, including the reduction of hydroperoxides, the quenching of free radicals, and the detoxification of xenobiotics [8]. The GSH-dependent antioxidant system consists of GSH and an array of functionally related enzymes, including glutathione S-transferase (GSH-ST), glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-Rd). GSH-ST is not only capable of conjugating a number of potentially toxic electrophilic xenobiotics to the nucleophilic GSH, but can also catalyze reactions to reduce peroxides. GSH-Px can reduce hydroperoxides as well as H₂O₂ but also oxidizes GSH. GSH-Rd then reduces oxidized glutathione (GSSG) to the GSH.

The liver is the primary site for total body GSH turn-over and accounts for over 90% of GSH inflow into the systemic circulation [8]. Since the liver plays a central role in the interorgan GSH homeostasis, liver dysfunction might affect endogenous production and utilization of GSH, and possibly further lead to dysregulation of the GSH-dependent antioxidant system. Previous studies have shown that patients with liver cirrhosis or HCC had decreased plasma GSH concentration [9–11] and GSH-dependent antioxidant enzyme activities [2,5,12]. However, the changes in oxidative stress and the GSH-dependent antioxidant system after HCC tumor resection have not been reported. While a previous study indicated that plasma GSH concentration was highly correlated with the intrahepatic level [13], whether the expression of GSH and its dependent antioxidant enzyme activities in plasma are correlated with their expression in tissues during the development of HCC is unknown. In order to better understand the role of GSH and its dependent antioxidant enzymes in the development of HCC and after tumor resection, we investigated the changes in oxidative stress, GSH status and its dependent antioxidant enzyme activities in HCC patients before and after tumor resection, and determined the association of oxidative stress with GSH and its dependent antioxidant enzyme activities in plasma and tissues.

Materials and Methods

Study design and sample size calculation

This was a cross-sectional study of HCC patients' pre- and post-resection oxidative stress, GSH status, and dependent antioxidant enzyme activities. Our group size was based on the requirement for the detection of a significant correlation coefficient of 0.4 between GSH in plasma and tumor tissue level with a power of 80% and a 2-sided test with an α of 0.05. The required sample size was at least 47 subjects. Our final recruitment number came to 60 HCC patients, which was greater than that determined in our original calculation.

Patients

Patients were recruited from the division of general surgery of Taichung Veterans General Hospital, Taiwan, if they were confirmed to have primary HCC [International Classification of Diseases 9, code 155.0] and were scheduled to undergo tumor resection. Patients did not

receive any treatment before surgery. Diagnosis and staging were confirmed by an oncologist and a pathologist based on the 7th edition of the American Joint Committee on Cancer staging system [14]. Patients were excluded if they were younger than 20 y or older than 80 y, were pregnant or lactating, were receiving chemotherapy, or had a history of cardiovascular or renal diseases. Each subject signed off on the informed consent document. This study was approved by the Institutional Review Board of Taichung Veterans General Hospital (IRB TCVGH No. CF13197).

Data collection and biochemical measurements

All subjects' age, gender, smoking and drinking habits, use of medications and clinical outcomes (i.e., severity of liver disease according to the Child-Pugh classification, hepatitis, cirrhosis or ascites) were recorded. Subjects' height and weight were measured and used to calculate their body mass index (BMI, kg/m²). Systolic and diastolic blood pressures (SBP and DBP) were measured after a resting period of at least 5 min.

Fasting blood samples were drawn and collected in vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA) containing an anticoagulant or no anticoagulant on the day before the tumor resection (pre-resection) and one month after the tumor resection (post-resection). Blood specimens were taken to measure serum albumin, alanine (ALT) and aspartate (AST) aminotransferases, serum creatinine, blood urea nitrogen (BUN) and α -fetoprotein. Hepatocellular tumor tissue and adjacent normal liver tissue were obtained at the time of surgical resection. Histological examinations and grade of HCC were confirmed by a pathologist and classified as well-, moderately-, and poorly-differentiated HCC. Resected tissues were homogenized on ice in 1 mL phosphate saline buffer (pH = 7.4). Homogenates were centrifuged at 13,000 rpm for 10 min at 4°C. The supernatants were frozen at -80°C until analysis. Plasma and tissue samples were used to analyze oxidative stress indicators [i.e., malondialdehyde (MDA), oxidized-low density lipoprotein-cholesterol (ox-LDL)], GSH, GSSG, trolox equivalent antioxidant capacity (TEAC) and antioxidant enzyme activities (i.e., GSH-Px, GSH-Rd, and GSH-ST).

Plasma MDA was measured by thiobarbituric acid reactive substances at an excitation wavelength of 515 nm and an emission wavelength of 555 nm using a fluorescence spectrophotometer according to a method described by Lapenna et al. [15]. A commercial kit (Merckodia AB, Sylveniusgatan 8A, SE-754 50 Uppsala, Sweden) was used to measure plasma ox-LDL level. TEAC was measured according to a previous method [16]. GSH and GSSG were measured using GSH and GSSG commercial kits (BioVision Incorporate, Milpitas, CA, USA). Activities of GSH-Px, GSH-Rd, and GSH-ST were determined using GSH-Px, GSH-Rd, and GSH-ST commercial kits (Cayman Chemical Company, Ann Arbor, MI, USA), respectively. All analyses were performed in duplicate.

Statistical analysis

The SAS statistical software package (version 9.3; Statistical Analysis System Institute Inc., Cary, NC, USA) was used for all data analyses. The Shapiro-Wilk test was performed to test the normal distribution. Demographic characteristics and biochemical data between the pre- and post-resection as well as between HCC tissue and adjacent normal tissue were compared using paired *t*-test or Wilcoxon signed rank test to determine any statistically significant differences. Chi-square or Fisher's exact tests were used in the analysis of categorical variables. Partial Spearman correlation coefficients (r_s) were used to analyze the associations of oxidative stress indicators with tumor grade, GSH, GSSG, GSH/GSSG ratio, TEAC, and GSH-dependent antioxidant enzyme activities in plasma and tissues after adjusting for age, gender, AST

level, cirrhosis, and smoking and drinking habits. Statistical significance was defined as a two-sided $p < 0.05$.

Results

[Table 1](#) shows the demographic and health characteristics of HCC patients. All patients' ages ranged from 30 to 79 years with a mean age of 59.2 ± 11.1 y. The severity of liver disease in 58 patients was Child-Pugh class A, and in 2 patients was Child-Pugh class B. Of the 60 patients, 82% of patients had a first-time diagnosis of HCC [new case] and 18% of patients had recurrent HCC. Twenty-eight patients were in cancer stage I and 32 patients were in cancer stage II, and more than two-thirds of patients had poorly differentiated HCC. The post-resection levels of BMI, AST, and α -fetoprotein in HCC patients significantly decreased, whereas BUN and creatinine significantly increased when compared with the corresponding pre-resection values. However, serum albumin, creatinine, and BUN levels were within normal range at pre- and post-resection.

[Table 2](#) shows the values of oxidative stress indicators, GSH, GSSG, and dependent antioxidant enzyme activities in plasma and tissues. Patients had significantly lower levels of plasma GSH, GSSG and GSH-Rd activities but higher plasma MDA, ox-LDL, and GSH-ST activity values before resection compared with the corresponding post-resection values. For tissue levels ([Table 3](#)), the GSH and TEAC levels as well as activities of GSH-Px were significantly higher in HCC tissue when compared with the adjacent normal tissue. Meanwhile, the MDA level in HCC tissue was significantly lower than that of the adjacent normal tissue.

The result of partial Spearman correlation coefficient showed no association between tumor histological grading and tissue GSH and oxidative stress (data not shown). [Table 4](#) shows significant partial correlation coefficients of oxidative stress with GSH, GSSG, and antioxidant capacities. The pre-resection plasma MDA level was significantly correlated with the pre-resection plasma GSH, pre-resection GSH/GSSG ratio, MDA level in HCC tissue adjacent normal tissue after adjusting for potential confounders. Pre-resection plasma ox-LDL level was significantly associated with pre-resection plasma GSH and GSSG levels. Pre-resection plasma GSH concentration was significantly associated with GSH level in HCC tissue and TEAC level in HCC tissue after adjusting for potential confounders. However, post-resection plasma MDA or ox-LDL had no significant association with post-resection plasma GSH, GSSG, GSH/GSSG ratio and GSH-dependent antioxidant enzyme activities following adjustment for potential confounders (data not shown).

Discussion

Carcinogenesis may progress or be enhanced if an imbalance between oxidative stress and antioxidant defense capacities occur. Our patients had increased oxidative stress (i.e., MDA and ox-LDL levels) and decreased GSH and GSSG levels before they underwent HCC resection; however, the expression of oxidative stress and GSH status was reversed after the HCC resection. This phenomenon supports the notion that increased oxidative stress is an important pathogenic mechanism of HCC [2,3,5], and suggests that GSH might be utilized and oxidized to GSSG in order to cope with increased oxidative stress during the development of HCC. A significant decrease in plasma GSH among HCC patients was also observed in a previous study [3]. In contrast to previous results indicating a decrease of GSH might be due to the increase in plasma GSSG level and GSH/GSSG ratio in HCC patients [3,17], the pre-resection plasma GSH/GSSG ratio in our HCC patients actually remained steady when compared to the post-resection level. Even though our HCC patients were under increased oxidative stress status and GSH-Rd activity was decreased before tumor resection, their GSH-Rd activities were

Table 1. Demographic and health characteristics of patients with hepatocellular carcinoma¹.

Parameters	Pre-resection [n = 60]	Post-resection [n = 60]
Age [y]	59.17 ± 11.10	
Male / Female	44 / 16	
BMI [kg/m ²]	23.93 ± 3.24*	23.38 ± 3.41
Blood pressure		
SBP [mmHg]	128.58 ± 18.98	130.11 ± 18.46
DBP [mmHg]	76.68 ± 12.63	77.74 ± 13.29
Serum ALT [U/L]	63.57 ± 58.73	54.80 ± 48.05
Serum AST [U/L]	66.88 ± 62.71*	40.28 ± 36.56
BUN [mg/dL]	13.80 ± 5.07*	16.38 ± 6.72
Serum albumin [g/dL]	4.05 ± 0.37	4.19 ± 0.31
Serum creatinine [mg/dL]	0.82 ± 0.25*	0.91 ± 0.29
α-fetoprotein [μg/L]	2992.98 ± 11464.46*	304.36 ± 1326.88
Cases [n, %]		
New	49 [81.7%]	
Recurrent	11 [18.3%]	
Stage at diagnosis [n, %]		
Stage I	28 [46.7%]	
Stage II	32 [53.3%]	
Histological grading [n, %]		
Well-differentiated [I]	1 [1.7%]	
Moderately-differentiated [II]	13 [21.7%]	
Poorly-differentiated [III]	46 [76.7%]	
Cirrhosis [n, %]		
Yes	45 [75%]	
No	15 [25%]	
Ascites [n, %]		
Yes	2 [3.3%]	
No	58 [96.7%]	
Hepatitis [n, %]		
No hepatitis	4 [6.7%]	
Hepatitis B	38 [63.3%]	
Hepatitis C	17 [28.3%]	
Co-hepatitis B and C	1 [1.7%]	
Smoking habit [n, %]		
Yes	13 [21.7%]	
No	47 [78.3%]	
Drinking habit [n, %]		
Yes	6 [10%]	
No	54 [90%]	

¹Values are means ± standard deviation. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen. *Values are significantly different from post-resection; *p* < 0.05.

doi:10.1371/journal.pone.0170016.t001

not been fully exhausted so GSSG could still be reduced to GSH, thereby preventing the accumulation of GSSG. That is to say, the balance of GSH/GSSG redox could be maintained and was not completely shifted to the oxidized state in our HCC patients. In the antioxidant

Table 2. Plasma reduced and oxidized, glutathione, indicators of oxidative stress, and antioxidant capacities in patients with hepatocellular carcinoma.

	Pre-resection [n = 60]	Post-resection [n = 60]
GSH [$\mu\text{mol/L}$]	38.86 \pm 26.15*	48.66 \pm 30.17
GSSG [$\mu\text{mol/L}$]	505.39 \pm 98.24*	589.74 \pm 119.92
GSH/GSSG ratio [$\times 10^{-2}$]	7.62 \pm 4.36	8.24 \pm 4.94
Oxidative stress indicators		
MDA [$\mu\text{mol/L}$]	1.01 \pm 0.28*	0.97 \pm 0.88
Oxidized-LDL [U/L]	37.64 \pm 8.00*	33.72 \pm 8.71
Antioxidant capacities		
TEAC [$\mu\text{mol/L}$]	4421.72 \pm 616.52	4593.42 \pm 496.29
GSH-Px [nmol/mL/min]	125.60 \pm 85.79	148.84 \pm 92.77
GSH-Rd [nmol/mL/min]	53.42 \pm 13.50*	66.99 \pm 15.32
GSH-ST [nmol/mL/min]	47.65 \pm 22.51*	38.85 \pm 16.27

Values are means \pm standard deviation. HCC, hepatocellular carcinoma; GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde; Oxidized-LDL, oxidized-low-density lipoprotein cholesterol; TEAC, trolox equivalent antioxidant capacity; GSH-Px, glutathione peroxidase; GSH-Rd, glutathione reductase; GSH-ST, glutathione S-transferase.

*Values are significantly from post-resection; $p < 0.05$.

doi:10.1371/journal.pone.0170016.t002

defense system, GSH-Px is considered to be the primary antioxidant enzyme, while GSH-ST serves as the secondary antioxidant enzyme. Our results revealed that the GSH-Px activity only slightly but not significantly decreased and GSH-ST activity was significantly increased before HCC resection, which implies that our patients tolerated the oxidative stress well. However, it is not known whether these properties of GSH, GSSG, and GSH-dependent antioxidant enzyme activities would persist if carcinogenesis progressed for a longer period.

In contrast to the expression of MDA and GSH in the circulation, we detected significantly lower MDA and higher GSH, TEAC, and GSH-Px activity in HCC tissue when compared to adjacent normal tissue. Similar findings were also observed in rat hepatoma tissues [18] and human HCC tissue [19,20] as compared to non-HCC tissues. High intracellular GSH levels could protect cell death and promote tumor growth while a depleted intracellular GSH level might cause the onset of apoptosis and cytotoxicity [21–24]. Even though large amounts of free radicals are generated by tumor cells during carcinogenesis, tumors could protect

Table 3. Reduced glutathione, oxidative stress indicator, and antioxidant capacities in hepatocellular carcinoma tissues and adjacent normal tissues.

	HCC tissue	Adjacent normal tissue
GSH [$\mu\text{mol/g}$ protein]	42.76 \pm 20.59*	29.17 \pm 14.92
Oxidative stress indicators		
MDA [$\mu\text{mol/g}$ protein]	0.46 \pm 0.50*	0.85 \pm 0.42
Antioxidant capacities		
TEAC [$\mu\text{mol/g}$ protein]	256.84 \pm 82.76*	201.29 \pm 58.38
GSH-Px [nmol/min/mg protein]	41.43 \pm 17.10*	31.07 \pm 10.95
GSH-Rd [nmol/min/mg protein]	61.05 \pm 35.73	55.23 \pm 94.66
GSH-ST [nmol/min/mg protein]	60.61 \pm 75.51	70.94 \pm 37.97

Values are means \pm standard deviation. HCC, hepatocellular carcinoma; GSH, reduced glutathione; MDA, malondialdehyde; TEAC, trolox equivalent antioxidant capacity; GSH-Px, glutathione peroxidase; GSH-Rd, glutathione reductase; GSH-ST, glutathione S-transferase.

*Values are significantly different from adjacent normal liver tissue; $p < 0.05$.

doi:10.1371/journal.pone.0170016.t003

Table 4. Partial Spearman’s correlation coefficients [r_s] of oxidative stress with glutathione, oxidized glutathione, and antioxidant capacities in plasma and tissues.

	Pre-resection plasma MDA [$\mu\text{mol/L}$]	Pre-resection plasma oxidized-LDL [U/L]	Pre-resection plasma GSH [$\mu\text{mol/L}$]
	<i>partial r_s¹</i>		
MDA [$\mu\text{mol/g protein}$]			
HCC tissue	0.64*	NS	NS
Adjacent normal tissue	0.47**	NS	NS
GSH [$\mu\text{mol/L}$]			
Pre-resection	0.35*	0.33*	– ²
GSH [$\mu\text{mol/g protein}$]			
HCC tissue	NS	NS	0.57*
GSSG [$\mu\text{mol/L}$]			
Pre-resection	NS	0.34*	–
GSH/GSSG/ ratio			
Pre-resection	0.34*	NS	–
TEAC [$\mu\text{mol/g protein}$]			
HCC tissue	NS	NS	0.63*

¹Values are partial correlation coefficient [r_s]. Adjusting for age, gender, aspartate aminotransferase level, cirrhosis, smoking and drinking habits.

* $p < 0.05$

** $p < 0.01$.

HCC, hepatocellular carcinoma; MDA, malondialdehyde; Oxidized-LDL, oxidized-low-density lipoprotein cholesterol; GSH, reduced glutathione; GSSG, oxidized glutathione; TEAC, trolox equivalent antioxidant capacity; NS, non-significance.

²Not test.

doi:10.1371/journal.pone.0170016.t004

themselves against increased oxidative stress through the elevation of intracellular GSH concentration. In addition to the overproduction of GSH that has been reported in breast or ovarian tumors [25–27], we also observed increased GSH concentration in hepatocellular tumor. The elevation of GSH level in HCC tissue might be contributed to the redistribution of GSH from plasma to HCC tissues since we observed a significant association between the GSH level in plasma and in HCC tissue. Although we did not evaluate HCC perfusion and tumor vascularization in the present study, primary HCC is known to be a typically and highly vascular malignant tumor through angiogenesis of hepatic artery and liver perfusion [28]. Thus, we speculate that hepatocellular tumor might be superior to its adjacent normal tissue in its ability to obtain GSH from plasma and decrease oxidative stress in order to facilitate tumor growth and invasion. In addition to the association between oxidative stress and GSH, the ratio of reduced and oxidized forms of GSH (GSH/GSSG) was used to critically evaluate oxidative stress in the cells [29]. The shifting of GSH/GSSG redox toward the oxidizing state would reduce cell proliferation and increase apoptosis [30]. Although we did not measure tissue GSSG level, the balance of GSH/GSSG redox seemed to be maintained in HCC tissue.

It worth noting that our results on the expression of oxidative stress, GSH status and its dependent enzymes in HCC tissue and adjacent normal tissue differ from those reported in the literature. In contrast to the findings of the present and previous studies [19,20], other results found an increase of MDA and a decrease of GSH levels in human HCC tissue compared to healthy or benign liver tissue [1,11,25,31]. Although we agree with the authors of previous studies who have suggested that reduced synthesis of GSH induced by liver dysfunction and increased oxidative stress might cause HCC tissue to lose its capacity to provide protection against oxidative impairment [1,11,25,31], this cannot explain why our HCC patients had lower MDA and higher GSH levels in HCC tissue when compared to its adjacent normal liver

tissue. Yang et al. [7] reported that well-differentiated HCC cell lines expressed much higher GSH-Rd enzyme activities than poorly differentiated HCC cell lines. Lee et al. [11] indicated that most poorly differentiated HCC had the lowest levels of GSH, GSSG and GSH/GSSG ratio when compared to well- and moderately-differentiated HCC tissues. More than two-thirds of the patients we recruited had poorly differentiated HCC while only one patient had well-differentiated HCC. In addition, no HCC patients were in stage III or IV based on the TNM system. Therefore, sample size disequilibrium of each differentiated group and degree of HCC stage might be possible reasons for us not detecting any significant associations of tumor grade with oxidative stress or GSH level. The factors underlying the discrepancy in the expression of oxidative stress and GSH status in HCC tissue reported among different studies requires further investigation.

The strength of this study was that most of the patients were new cases, so this effectively eliminated the potential confounding effects of changes or modifications that occur in patients who were diagnosed a relatively long time ago. The second strength was that our sample size was 2.5 ~ 4.6 times larger than those analyzed in previous studies [1,11,25,31], which allowed more powerful statistical inferences. Another strength was that this was the first study to assess the changes of oxidative stress, GSH status, and its dependent antioxidant capacities before and after hepatocellular tumor resection. However, there were some limitations in this study. We could not obtain an adequate size of HCC and adjacent normal tissues in some patients, so some tissue parameters [i.e., GSSG and ox-LDL] could not be measured, which may have decreased the statistical power to observe the association between the level in plasma and tissues. The second limitation was possibly the timing for the post-resection and pre-resection evaluations, which may have been too close together. The post-resection data might therefore not be truly representative of the characteristics that would be observed under tumor-free conditions. The third possible limitation was that dietary intake was not recorded; it is not known that diet may influence oxidative stress status.

Conclusions

The data herein indicate that although HCC patients had increased oxidative stress and decreased GSH-dependent antioxidant capacity in the circulation before tumor resection, hepatocellular tumor had increased GSH and TEAC levels as well as activities of GSH-Px which might protect itself against increased oxidative stress. It would be clinically valuable to know how hepatocellular tumors obtain more GSH from the circulation than adjacent normal tissue since there is currently no effective medical therapy for HCC.

Acknowledgments

We thank Dr. Yung-Po Liaw (Department of Public Health, Chung Shan Medical University) for his professional consultation in statistical analyses. We really appreciate patients for their participation and thank the technician and the nurses at Taichung Veterans General Hospital for providing expert assistance in collecting blood samples.

Author Contributions

Conceptualization: YCH.

Formal analysis: YCH PTL.

Investigation: SBC HTL CYL.

Methodology: YCH SBC.

Supervision: YCH.

Validation: SYC YCH.

Writing – original draft: YCH.

References

1. Czczot H, Scibior D, Skrzycki M, Podsiad M. Glutathione and GSH-dependent enzymes in patients with liver cirrhosis and hepatocellular carcinoma. *Acta Biochim Pol.* 2006; 53: 237–241. PMID: [16404476](#)
2. Lin CC, Yin MC. B vitamins deficiency and decreased anti-oxidative state in patients with liver cancer. *Eur J Nutr.* 2007; 46: 293–299. doi: [10.1007/s00394-007-0665-8](#) PMID: [17571208](#)
3. Tsai SM, Lin SK, Lee KT, Hsiao JK, Huang JC, Wu SH, et al. Evaluation of redox statuses in patients with hepatitis B virus-associated hepatocellular carcinoma. *Ann Clin Biochem.* 2009; 46: 394–400. doi: [10.1258/acb.2009.009029](#) PMID: [19641006](#)
4. Yuan L, Kaplowitz N. Glutathione in liver diseases and hepatotoxicity. *Mol Aspects Med.* 2009; 30: 29–41. doi: [10.1016/j.mam.2008.08.003](#) PMID: [18786561](#)
5. Yahya RS, Ghanem OH, Foyouh AA, Atwa M, Enany SA. Role of interleukin-8 and oxidative stress in patients with hepatocellular carcinoma. *Clin Lab.* 2013; 59: 969–976. PMID: [24273918](#)
6. Miura Y, Kozuki Y, Yagasaki K. Potentiation of invasive activity of hepatoma cells by reactive oxygen species is mediated by autocrine/paracrine loop of hepatocyte growth factor. *Biochem Biophys Res Commun.* 2003; 305: 160–165. PMID: [12732211](#)
7. Yang LY, Chen WL, Lin JW, Lee SF, Lee CC, Hung TI, et al. Differential expression of antioxidant enzymes in various hepatocellular carcinoma cell lines. *J Cell Biochem.* 2005; 96: 622–631. doi: [10.1002/jcb.20541](#) PMID: [16052474](#)
8. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. *J Nutr.* 2004; 134: 489–492. PMID: [14988435](#)
9. Bianchi G, Bugianesi E, Ronchi M, Fabbri A, Zoli M, Marchesini G. Glutathione kinetics in normal man and in patients with liver cirrhosis. *J Hepatol.* 1997; 26: 606–613. PMID: [9075668](#)
10. Bianchi G, Brizi M, Rossi B, Ronchi M, Grossi G, Marchesini G. Synthesis of glutathione in response to methionine load in control subjects and in patients with cirrhosis. *Metabolism.* 2000; 49: 1434–1439. doi: [10.1053/meta.2000.16554](#) PMID: [11092507](#)
11. Lee KT, Tsai SM, Wang SN, Lin SK, Wu SH, Chuang SC, et al. Glutathione status in the blood and tissues of patients with cirrus-originated hepatocellular carcinoma. *Clin Biochem.* 2007; 40: 1157–1162. doi: [10.1016/j.clinbiochem.2007.06.012](#) PMID: [17706189](#)
12. Abou Ghalia AH, Fouad IM. Glutathione and its metabolizing enzymes in patients with different benign and malignant diseases. *Clin Biochem.* 2000; 33: 657–662. PMID: [11166013](#)
13. Griffith OW, Meister A. Glutathione: Interorgan translocation, turnover, and metabolism. *Proc Natl Acad Sci USA.* 1979; 76: 5606–5610. PMID: [42902](#)
14. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A, editors. *AJCC cancer staging manual.* 7th ed. New York: Springer; 2010
15. Lapenna D, Ciofani G, Pierdomenico SD, Giamberardino MA, Cuccurullo F. Reaction conditions affecting the relationship between thiobarbituric acid reactivity and lipid peroxides in human plasma. *Free Radic Biol Med.* 2001; 31: 331–335. PMID: [11461770](#)
16. Arnao MB, Cano A, Hernandez-Ruiz J, Garcia-Canovas F, Acosta M. Inhibition by L-ascorbic acid and other antioxidants of the 2,2'-azino-bis[3-ethylbenzthiazoline-6-sulfonic acid] oxidation catalyzed by peroxidase: a new approach for determining total antioxidant status of foods. *Anal Biochem.* 1996; 236: 255–261. PMID: [8660502](#)
17. Lu SC. Regulation of glutathione synthesis. *Mol Aspects Med.* 2009; 30:42–59. doi: [10.1016/j.mam.2008.05.005](#) PMID: [18601945](#)
18. Marengo B, Ciucis CD, Ricciarelli R, Romano P, Passalacqua M, Marinari UM, et al. DNA oxidative damage of neoplastic rat liver lesions. *Oncology Reports.* 2010; 23: 1241–1246. PMID: [20372836](#)
19. Huang ZZ, Chen C, Zeng Z, Yang H, Oh J, Chen L, et al. Mechanism and significance of increased glutathione level in human hepatocellular carcinoma and liver regeneration. *FASEB J.* 2001; 15: 19–21. doi: [10.1096/fj.00-0445fje](#) PMID: [11099488](#)
20. Jünger C, Cheng B, Gehrke R, Schmitz V, Nischalke HD, Ramakers J, et al. Oxidative stress is increased in human liver tissue adjacent to hepatocellular carcinoma. *Hepatology.* 2004; 39: 1663–1672.

21. Hammond CL, Lee TK, Ballatori N. Novel roles for glutathione in gene expression cell death, and membrane transport of organic solutes. *J Hepatol.* 2001; 34: 946–954. PMID: [11451183](#)
22. Benlloch M, Ortega A, Ferrer P, Segarra R, Obrador E, Asensi M, et al. Acceleration of glutathione efflux and inhibition of γ -glutamyltranspeptidase sensitize metastatic B16 melanoma cells to endothelium-induced cytotoxicity. *J Biol Chem.* 2005; 280: 6950–6959. doi: [10.1074/jbc.M408531200](#) PMID: [15561710](#)
23. Traverso N, Ricciarelli R, Nitti M, Marengo B, Furfaro AL, Pronzato MA, et al. Role of glutathione in cancer progression and chemoresistance. *Oxid Med Cell Longev.* 2013; 2013: 1–10.
24. Ortega AL, Mena S, Estrela JM. Glutathione in cancer cell death. *Cancers* 2011; 3:1285–1310. doi: [10.3390/cancers3011285](#) PMID: [24212662](#)
25. Azza H, Abou G, Imam MF. Glutathione and its metabolizing enzymes in patients with different benign and malignant diseases. *Clin Biochem.* 2000; 33: 657–662. PMID: [11166013](#)
26. Perquin M, Oster T, Maul A, Froment N, Untereiner M, Bagrel D. The glutathione-related detoxification system is increased in human breast cancer in correlation with clinical and histopathological features. *J Cancer Res Clin Oncol.* 2001; 127: 368–374. PMID: [11414197](#)
27. Kumaraguruparan R, Subapriya R, Viswanathan P, Nagimi S. Tissue lipid peroxidation and antioxidant status in patients with adenocarcinoma of the breast. *Clin Chim Acta.* 2002; 325: 165–170. PMID: [12367782](#)
28. Asayama Y, Yoshimitsu K, Nishihara Y, Irie H, Aishima S, Taketomi A, et al. Arterial blood supply of hepatocellular carcinoma and histologic grading: radiologic-pathologic correlation. *AJR Am J Roentgenol* 2008; 190: W28–W34. doi: [10.2214/AJR.07.2117](#) PMID: [18094269](#)
29. Chai YC, Ashraf SS, Kutank Ro, Johnston RB Jr, Thomas JA. S-thiolation of individual human neutrophil proteins including action by stimulation of the respiratory burst: evidence against a role for glutathione disulfide. *Arch Biochem Biophys.* 1994; 310: 273–281. doi: [10.1006/abbi.1994.1167](#) PMID: [8161216](#)
30. Shen CK. Cellular thiols and redox-regulated signal transduction. *Curr Top Cell Regul.* 2000; 36: 1–30. PMID: [10842745](#)
31. Abel S, De Kock M, van Schalkwyk DJ, Swanevelder S, Kew MC, Gelderblom WC. Altered lipid profile, oxidative status and hepatitis B virus interactions in human hepatocellular carcinoma. *Prostaglandins Leukot Essent Fatty Acids.* 2009; 81:391–399. doi: [10.1016/j.plefa.2009.08.003](#) PMID: [19782547](#)