RESEARCH ARTICLE

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Inverse Association between Glucose–6–Phosphate Dehydrogenase Deficiency and Hepatocellular Carcinoma

Maria Pina Dore[1,2]*, Gianpaolo Vidili[1], Giuseppina Marras[1], Silas Assy[1], Giovanni Mario Pes[1]

Abstract

Background: Studies in experimental models and humans suggest that glucose–6–phosphate dehydrogenase (G6PD) deficiency, an inherited condition, may be inversely related to hepatocellular carcinoma (HCC). We tested this hypothesis in a large cohort of Sardinian patients. **Methods:** A case-control study was performed using data from 11,143 records of patients who underwent upper endoscopy between 2002 and 2017. Gender, age, G6PD status and information regarding the presence of HCC, were recorded. Cases (HCC positive) and controls (HCC negative) were compared for the presence of G6PD deficiency adjusting for major HCC risk factors using logistic regression. **Results:** Overall, 114 HCC cases and 11,029 controls were identified. G6PD deficiency was detected in 11.5% of study participants, and was associated with a reduced risk of HCC [odds ratio (OR); 0.451; 95% confidence interval (CI), 0.207−0.982] after adjusting for all covariates. Factors significantly associated with HCC were cirrhosis (OR, 23.30; 95% CI, 11.48−47.25), diabetes (OR, 2.396; 95% CI, 1.449−3.963), among infection hepatitis HBV with an OR of 2.326, age ≥65 years (OR, 1.941; 95% CI, 1.234−2.581) and male gender (OR, 1.611; 95% CI, 1.006−3.081). **Conclusions:** Our study revealed a significant inverse association between G6PD deficiency and risk of HCC. These findings need to be confirmed in further studies.

Keywords: Hepatocellular carcinoma- glucose–6–phosphate dehydrogenase- liver disease- risk stratification

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Introduction

Hepatocellular carcinoma (HCC), the fifth most frequently diagnosed malignancy worldwide, is highly aggressive with a median survival of 6 to 20 months (Jemal et al., 2011; Colombo et al., 1991). Incidence and prevalence of HCC vary across different countries and even within the same geographic region among racial and ethnic groups, depending on exposure to various risk factors (El–Serag, 2002). Chronic liver disease and cirrhosis of any etiology are the major risk factors associated with development of HCC (Tsukuma et al., 2007). Additional risk factors include heavy alcohol consumption, smoking, diabetes, obesity, exposure to food contaminated with aflatoxin, and non–alcoholic fatty liver disease (El–Serag et al., 2007). An increased understanding of factors influencing the HCC risk would lead to more effective recommendations for surveillance in specific subgroups of patients (Yu, 2016).

Glucose–6–phosphate dehydrogenase (G6PD) is a housekeeping enzyme expressed in most cells. Over the past few decades various in vitro and in vivo studies suggested that G6PD deficiency may be protective against HCC, possibly due to downregulation of pentose phosphate pathway (PPP) which is preferentially used by cancer cells instead of oxidative phosphorylation (Kowalik et al., 2017; Pascale et al., 1990; Simile et al., 2001).

The frequency of G6PD deficiency in the Sardinian population is one of the highest in the world, ranging between 12% and 24% (De Vita et al., 1989; Fiorelli et al., 1990). Patients with this inherited condition may experience hemolysis after exposure to fava beans and/or certain drugs. Identification of G6PD deficiency allows patient education regarding unsafe medications and foods in order to prevent episodes of hemolysis. For this reason, in Sardinia this condition is tested routinely since childhood.

In this study the association between G6PD deficiency and risk of HCC was analysed in a large number of patients from Northern Sardinia.

Materials and Methods

Study population

This was a case-control study. Data recorded a priori in a large database of in– and out–patients undergoing upper endoscopy from January 2002 to January 2017 were analysed. Patients were referred to the endoscopy

*[1]Dipartimento di Medicina Clinica e Sperimentale, University of Sassari, Sassari 07100, Italy, [2]Baylor College of Medicine, 77030 Houston, Texas, USA. *For Correspondence: mpdore@uniss.it*

service by family physicians and/or specialists for any reason (dyspeptic or reflux symptoms, varices detection and assessment of hemorrhage risk, follow-up, etc.).

Setting

The endoscopy service is part of a teaching Hospital "Clinica Medica" at the Department of Clinical and Experimental Medicine (University of Sassari, Northern Sardinia, Italy), a tertiary referral center which provides care for patients with gastrointestinal problems including most adult cases of liver diseases and HCC.

Measures

At the time of upper endoscopy, a trained gastroenterologist collected a detailed clinical history from each patient. Only definitive disease diagnoses confirmed with a written report and/or a disease code (exempting patients from any co-payment) by the specific specialist were recorded as comorbidities.

For each patient, information regarding age, gender, marital status, G6PD status, presence of liver disease and its etiology, type 1 and 2 diabetes, were retrieved. For patients who underwent multiple EGDs within the study period, only the results from the last endoscopy were included.

The presence of HCC was determined by dynamic contrast-enhanced imaging studies and or histopathological features in tissue specimens obtained by needle biopsy according to major liver Societies guidelines followed in clinical routine (Bruix et al., 2011; European Association For The Study Of The Liver 2012).

Because of the high frequency of G6PD deficiency in Sardinia, the enzyme activity is routinely measured since early childhood. The quantitative biochemical assay is based on the G6PD/6GPD ratio in erythrocytes according to the official WHO recommended methodology (Mosca et al., 1996). Genotyping analysis was not available in G6PD deficient patients.

Statistical analysis

The distribution of age, gender, etiological factors for liver disease, diabetes history and marital status were descriptively compared between cases and controls. Type 1 and 2 diabetes were pooled together since they are both risk factors for HCC regardless of the initial etiology. Logistic regression was used to determine the association of HCC with G6PD status calculating adjusted odds ratios (ORs) and their 95% confidence intervals (CI). The age of patients was recoded into a binary variable by considering the age of 65 years as a cut-off. Patients with total and partial G6PD deficiency were pooled together. The main effect of variables was assessed first by running a full model including all covariates simultaneously, then a stepwise backward elimination procedure was used with a removal threshold of 0.05, to find the minimum number of significant variables describing the data.

Since gender and age could modify the association of G6PD status with HCC, their interaction with G6PD was tested by entering their product terms into the regression model. For each covariate, the regression coefficients β and their standard error (SE) were calculated, as well as the ORs and their 95% CI using the Wald formula (95% CI = $OR^{1\pm ß/SE}$). All statistical analyses were performed using SPSS statistical software (version 16.0, Chicago, IL, USA). P–values lower than 0.05 were considered statistically significant.

Results

A total of 11,143 clinical records (4,266 men, mean age 52.8 ± 17.5 years) (Table 1), were analyzed. In the total study population, the proportion of patients with G6PD deficiency was 11.5% (1,280/11,143) similar to the previously reported frequency in the general population of Northern Sardinia (De Vita et al., 1989; Fiorelli et al., 1990). There were 114 HCC cases all associated with hepatitis or cirrhosis. HCC cases were older and more likely male than controls (Table 1). As expected, the distribution of risk factors, such as liver diseases of any etiology and diabetes, were more prevalent in HCC cases than in controls.

Distribution of marital status was similar in HCC cases and controls. The proportion of G6PD deficiency was 7.0% in HCC cases and 11.5% in controls patients (8/114 versus 1272/11,029).

Unconditional logistic regression showed that G6PD deficiency was inversely associated with HCC in the full model (Table 2), with an OR of 0.446 (95% CI, 0.203−0.981). This trend persisted after adjusting for all covariates through the stepwise backward procedure, with a risk of HCC 55% lower in patients with G6PD

Table 1. Descriptive Characteristics of Hepatocellular Carcinoma (HCC) Cases and Controls

Characteristics	HCC Cases	Controls	Total
No. of patients	114	11,029	11,143
Gender (M/F)	77/37	4189/6840	4266/6877
Age at endoscopy (mean ± SD, years)	66.7 ± 10.3	52.6 ± 17.4	52.8 ± 17.5
Liver disease			
None	0 (0.0%)	9842 (89.2%)	9842 (88.3%)
Hepatitis	9 (7.9%)	817 (7.4%)	826 (7.4%)
Cirrhosis	105 (92.1%)	370 (3.4%)	475 (4.3%)
Diabetes mellitus	34 (19.8%)	819 (7.4%)	853 (7.7%)
Liver disease etiology [a]			
HCV	74 (64.9%)	648 (5.9%)	722 (6.5%)
HBV	18 (15.8%)	149 (1.4%)	167 (1.5%)
Alcohol	18 (15.8%)	138 (1.3%)	156 (1.4%)
Multiple etiology	9 (7.9%)	152 (1.4%)	161 (1.4%)
Others	9 (7.9%)	208 (1.9%)	217 (1.9%)
Marital status [b]			
Single	16 (14.0%)	3055 (27.8%)	3071 (27.6%)
Married	81 (71.1%)	6668 (60.6%)	6749 (60.7%)
Widow	13 (11.4%)	902 (8.2%)	915 (8.2%)
Divorced	4 (3.5%)	376 (3.4%)	376 (3.4%)
G6PD			
Normal	106 (93.0%)	9757 (88.5%)	9863 (88.5%)
Deficient	8 (7.0%)	1272 (11.5%)	1280 (11.5%)

[a]Etiology factors are expressed as percentage of the total number of patients with liver disease; [b]Unavailable in 6 patients.

Table 2. Logistic Regression in 11,143 Patients for Hepatocellular Carcinoma (HCC). The dependent variable is the presence/absence of HCC (yes/no).

Covariates	Model 1[a]			Model 2[b]		
	ORs	95% CI of OR	p-value	ORs	95% CI of OR	p-value
Age (median split)						
<64	1	–	–	1	–	–
≥65	1.682	(1.045 – 2.708)	0.032	1.941	(1.234 – 2.581)	0.004
Gender						
Women	1	–	–	1	–	–
Men	1.677	(1.010 – 2.783)	0.045	1.611	(1.006 – 3.081)	0.047
G6PD deficiency						
No	1	–	–	1	–	–
Yes	0.446	(0.203 – 0.981)	0.045	0.451	(0.207 – 0.982)	0.045
G6PD deficiency x age	1.257	(0.210 – 7.528)	0.802	–	–	–
G6PD deficiency x gender	0.591	(0.119 – 2.941)	0.521	–	–	–
Liver disease						
Hepatitis	1	–	–	1	–	–
Cirrhosis	24.3	(11.93 – 49.45)	<0.0001	23.3	(11.48 – 47.25)	<0.0001
Etiology						
HCV	1.63	(0.956 – 2.779)	0.073	1.752	(1.103 – 2.781)	0.017
HBV	2.141	(1.082 – 4.234)	0.029	2.326	(1.234 – 4.383)	0.009
Alcoholic	0.646	(0.328 – 1.273)	0.207	–	–	–
Diabetes mellitus						
No	1	–	–	1	–	–
Yes	1.385	(0.856 – 2.243)	0.185	2.396	(1.449 – 3.963)	0.001
Marital status						
Single	1	–	–	–	–	–
Married	1.623	(0.869 – 3.033)	0.129	–	–	–
Widowed	1.312	(0.542 – 3.176)	0.548	–	–	–
Divorced	0.741	(0.223 – 2.456)	0.624	–	–	–
Constant	0.003		0.956	0.003		0.957

[a]All variables simultaneously (full model); [b]Stepwise backward elimination.

deficiency compared with patients with normal G6PD (OR = 0.451, 95% CI 0.207–0.982). In this model, the variables "alcohol", "marital status" and the two interaction terms of G6PD with age and gender disappeared as they did not improve model fitting. According to literature, older age, male gender, presence of cirrhosis, presence of diabetes, as well as HCV and HBV infection were confirmed to be independent risk factors.

Discussion

In this case-control study the association of HCC with G6PD status was assessed by unconditional logistic regression, adjusting for gender, age, and marital status as well as for well–defined major risk factors for HCC such as hepatitis C and B viruses, alcohol consumption, cirrhosis of any etiology, and diabetes. Patients with G6PD deficiency showed a 55% risk reduction for HCC compared with patients with normal G6PD activity. The risk reduction was independent of age and gender, since the interactions between G6PD deficiency and age and gender were not statistically significant.

Hepatocellular carcinoma is associated with a high mortality, with more than 700,000 deaths per year in the world (El–Serag et al., 2007; Sherman et al., 2012). Clinical manifestations are often confused with the findings of the underlying liver disease, delaying diagnosis and affecting therapeutic intervention and long-term prognosis.

Surveillance programs with frequent monitoring including semi-annual intervals by imaging procedures and measurements of alpha-fetoprotein, are used to detect HCC at an early stage (Sherman et al., 2012; El–Serag et al., 2008). Surveillance recommendations are based on the incidence of HCC in a specific group of patients with liver disease. The American Association of Liver Diseases recommends surveillance in patients with chronic HBV infection; in Asian, African and Caucasian HBV carriers; in patients with a family history of HCC, and in all patients with cirrhosis of any etiology (Sherman et al., 2012; El–Serag et al., 2008). Surveillance may improve outcome if the risk of HCC can be predicted in the individual patient.

In the past half century, several epidemiological

investigations in Sardinia have suggested a role of G6PD deficiency on cancer (Kowalik et al., 2017; Dore et al., 2016). The frequency of G6PD deficiency in this region ranges between 12 and 24% according to different island areas (De Vita et al., 1989; Fiorelli et al., 1990), making Sardinians an ideal population to test this hypothesis. The overall frequency of G6PD in the cohort investigated was similar to the average frequency found in previous studies in the Sardinian population (Pes et al., 2017). This makes unlikely an under– or over–estimation of the enzyme defect in the study participants.

A recent review of Kowalik et al. stressed the pivotal role of PPP in the proliferation of hepatocellular carcinoma cells, and suggested that induced downregulation of G6PD may reduce liver carcinogenesis (Kowalik et al., 2017). In fact, G6PD deficiency produces a chronic nicotinamide–adenine dinucleotide phosphate (NADPH) shortage and thus the rate of operation of the oxidative branch of PPP may be reduced (Kowalik et al., 2017; Luzzatto et al., 2001). This, in turn, may reduce superoxide anion production and other free radicals, including nitric oxide which promotes DNA mutations and upregulates pro-inflammatory cytokines synthesis involved in carcinogenesis (Shen et al., 2015). Viewed from this perspective, the down–regulation of PPP may act similarly to some anticancer drugs capable of driving glucose into the aerobic glycolytic pathway. Additional mechanisms of protection provided by G6PD deficiency may be related to the reduced activation of chemical carcinogens (Pascale et al., 1987; Pascale et al., 1990), inhibition of apoptosis, (Hong et al., 2014), and induction of angiogenesis (Shen et al., 2015). These findings obtained in humans are consistent with the delayed liver cancer development in rodents induced by the selective G6PD inhibitor dehydro-epiandrosterone (DHEA) (Ho et al., 2008).

In our study G6PD deficiency was associated with a significant reduction of HCC even after adjusting for gender, age, marital status, liver disease and diabetes.

Several observational studies have reported that use of statin (Ampuero et al., 2015), coffee (Bravi et al., 2013), white meat and fish (Fedirko et al., 2013), omega–3 fatty acids (Huang et al., 2015), vitamin E (Zhang et al, 2012), and vegetables consumption (Bamia et al., 2015), are associated with a reduced risk of HCC, ranging from –18% to –48%. Compared to all these factors in our study G6PD deficiency was able to reduce the HCC risk to an even greater extent.

Some limitations in this study may be the lack of molecular genotyping for patient with biochemically identified G6PD deficiency, precluding the possibility, in the case of women, to discriminate between homozygotes and heterozygotes, and therefore to test the association of HCC with total or partial G6PD deficiency separately. However, the lack of more detailed analysis would not have changed the overall findings.

In conclusion, this study suggests that a loss–of–function mutation in a ubiquitous gene such as G6PD can reduce the risk of HCC. This finding is in line with the emerging hypothesis that G6PD deficiency has a broader biological role in carcinogenesis (Kowalik et al., 2017; Stanton et al., 2012), and may open new windows for molecular treatment of HCC. For example, when the genetic sequence of a particular gene is known it is possible to inactivate it by synthesizing an "antisense" oligonucleotide complementary to the messenger RNA (mRNA) produced by that gene (Callegari et al., 2015). Recently, gene antisense therapy for HCC with oligonucleotide targeting some proteins overexpressed during liver carcinogenesis has been proposed. These include antisense-miRNA-21 and other anti-miRNA in vitro (Devulapally et al., 2016); TGF-beta2 in experimental animals (Maggard et al., 2001), and Mcl-1 in humans (Sieghart et al., 2006). Further prospective studies are needed to confirm the inverse association between G6PD deficiency and HCC. Systematic determination of G6PD status in patients with liver disease may eventually result in new risk stratification systems and improvements in surveillance protocols for HCC.

Disclosure statement

The authors declare that they have no conflicts of interest.

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