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ORIGINAL ARTICLE

Lack of association of *GSTT1* and *GSTP1* genes methylation and their expression profiles with risk of NAFLD in a sample of Iranian patients

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Summary Reactive oxygen species can affect many cellular functions through protein oxidation or initiation of the lipid peroxidation cascade that can lead to non-alcoholic fatty liver disease (NAFLD), characterized by significant lipid deposition in the hepatocytes of patients with no history of excess alcohol intakes. The present study aimed to analyze the methylation status of the antioxidative stress genes *GSTT1* (glutathione S-transferase theta-1) and *GSTP1* (glutathione S-transferase pi-1), and their expression profiles, in a sample population of patients with NAFLD living in South-East Iran.

Patients and methods: Peripheral blood samples were obtained from 80 NAFLD patients and 80 healthy controls. Promoter methylation of the *GSTT1* and *GSTP1* genes were analyzed by methylation-specific polymerase chain reaction (MS-PCR). Expression profiles of these genes were also examined by quantitative real-time PCR analysis.

Results: Promoter methylation of the *GSTT1* gene was detected in 86.2% of cases and in 91.2% of controls and, of the *GSTP1* gene, in 88.8 and 87.5% of cases and controls, respectively. Promoter methylation of *GSTT1* and *GSTP1* was not statistically different in cases compared with healthy controls. Similarly, mRNA expression levels showed no statistically significant variations between healthy individuals and patients with NAFLD.

Conclusion: Our findings indicate no association between methylation status and expression profiles of *GSTT1* and *GSTP1* genes and NAFLD. This is the first report to assess such associations in a sample of the Iranian population.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common forms of liver disorder in both the developed and developing worlds [1]. However, there are geographical variations in the prevalence of the disease ranging from 10—24% in diverse populations [2]. According to the present data, NAFLD may be considered the most common cause of liver disease in the Iranian population [3].

NAFLD is defined as the presence of fat in the liver parenchyma despite a lack of significant alcohol intakes. It accounts for a broad spectrum of disorders, ranging from the simple presence of fat in the liver to related inflammatory fibrosis and non-alcoholic steatohepatitis (NASH). It is also suspected of being one of the etiologies in cryptogenic cirrhosis and hepatocellular carcinoma (HCC) [4,5]. NAFLD is a well-known symptom of the metabolic syndrome, hyperglycemia, central obesity, type 2 diabetes, hypertriglyceridemia and arterial hypertension [6]. Patients with NAFLD risk factors may progress to hepatic steatosis, and a minority may go on to develop more advanced diseases such as NASH, cirrhosis and HCC [7]. Recently, patients with hypothalamic/pituitary dysfunction, excessive weight gain, dyslipidemia and impaired glucose tolerance were also found to have progressive NAFLD [8].

Clearly, environmental and genetic factors play significant roles in the etiology of NAFLD [9,10]. Furthermore, epigenetic mechanisms, such as DNA methylation, also have significant roles in the regulation of gene expression [11,12]. Variations in DNA methylation profile could be used to verify differences in transcription-machinery function to locate promoter regions and begin transcription. Such covalent alteration leads to constant gene silencing, which is involved in the pathogenesis of various diseases [13—15].

Glutathione S-transferases (GSTs) are a superfamily of proteins that participate in phase II detoxification [16]. In humans, GST enzymes are divided into five subclasses: alpha (α); mu (μ); pi (π); theta (θ); and zeta (ζ). All of them participate in the metabolism of endogenous and exogenous carcinogenic agents, such as environmental carcinogens, reactive oxygen species and chemotherapeutic agents, by catalyzing reactions between glutathione and electrophilic compounds [17,18].

GSTT1 (glutathione S-transferase theta-1) belongs to the θ class, and is located on chromosome 22 [19]; its products are involved in the activation of detoxification reactions and catalysis of the conjugation of industrial chemicals with glutathione [20]. A polymorphism characterized by deletion of nearly the entire gene has led to the absence of the GSTT1 protein [21]. Furthermore, some *GSTT1* deletions can stop detoxification of some GSTT1 substrates that are either carcinogenic or highly toxic compounds [22]. *GSTP1* (glutathione S-transferase pi-1) belongs to the pi class, and is located on chromosome 11q13. It encodes functionally different GSTP1 variant proteins that are thought to be involved in xenobiotic metabolism, and to play a role in susceptibility to cancer and other diseases [23]. The most widely studied polymorphism at this locus is Ile105Val, which encodes for a protein with changed catalytic activity [24]. Khan et al. [25] showed that the interaction of GSTs with the variant genotype of manganese superoxide dismutase (MnSOD), which detoxifies free radicals and cytochrome P450 2E1, increased several fold in alcoholic liver cirrhosis compared with non alcoholic controls.

Epigenetic regulation of GST expression by DNA methylation has not been investigated in NAFLD, and there are no data on the association between regulation of these genes and NAFLD risk. Thus, there is a need for further studies to elucidate the epigenetic mechanisms involved in the progression of liver disease. The present study, for the first time, examines the relationship between *GSTP1* and *GSTT1* gene methylation and expression profiles in patients with NAFLD in a sample of the Iranian population.

Patients and methods

Study subjects and clinical tests

A total of 80 NAFLD and 80 healthy (without NAFLD) controls provided samples collected at the Ali-ebne-Abi-Taleb Hospital in Zahedan, Iran, during 2009—2010. The study was approved by the ethics committee of Zahedan University of Medical Sciences, and all studied samples were obtained with informed consent and from biochemical tests. Patients were excluded if they had other known causes of liver disease, including viral hepatitis B or C, hemochromatosis, Wilson disease, auto-immune liver diseases, a history of alcohol consumption of >100 g/week and chronic drug use. Individuals who were overweight (defined as a body mass index [BMI] > 25 kg/m^2), had type 2 diabetes, were hyperlipidemic and had abnormal liver function tests were included in the study.

Laboratory assays included fasting blood glucose, insulin, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, iron, total iron-binding capacity (TIBC), ferritin, ceruloplasmin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), billirubin, hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HBcAb), liver kidney microsomal type-1 (LKM-1) antibody, hepatitis C virus (HCV) antibody, antismooth muscle antibody, antimitochondrial antibody and antinuclear antibody (ANA) tests, with samples collected after a 12-hour overnight fast. In addition, hepatic ultrasonography was performed in all participants by an experienced radiologist, who was blinded to the subjects' clinical details. Diagnosis of NAFLD was performed according to clinical setting, and ultrasound and laboratory findings. No liver biopsies were taken out of concern for the patients. Control subjects were from the Zahedan population and had volunteered to participate in the project; all had normal blood pressure, lipid profile, blood glucose, BMI, waist circumference and liver function tests, and no history of systemic disease (Table 1). Analyses of gene methylation in the laboratory were carried out in parallel for both cases and controls.

DNA extraction and methylation analysis

Blood samples were collected in tubes containing EDTA and kept at −80° C until analysis. Two mL of blood were used for extraction of genomic DNA, from which 2 μg were used for bisulfate treatment, as described elsewhere [26].