Note

Relationship between Plasma Folate and Homocysteine Concentrations in Alcoholics According to Liver Enzyme Activity

Bogdan CYLWIK¹, Lech CHROSTEK¹, Marta DANILUK², Zbigniew SUPRONOWICZ²
and Maciej SZMITKOWSKI¹

¹Department of Biochemical Diagnostics, Medical University, Białystok, Waszyngtona 15A,
15–274 Białystok, Poland

²Department of Detoxification, Psychiatric Hospital, Choroszcz, Poland

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Summary The aim of this study was to evaluate the relationship between folate and homocysteine levels in alcoholics taking into consideration the liver enzyme activity as sensitive markers of hepatocellular injury. Folate and homocysteine concentrations did not differ between alcoholics classified according to the liver enzyme activity. The association between folate and homocysteine levels exists in the alcoholics with normal liver enzyme activity and in the controls. Therefore, we concluded that before the liver hepatocellular injury due to alcohol abuse, the correlation between folate and homocysteine concentrations in alcoholics exists as in the healthy controls. In the presence of hepatocellular injury, the association disappears.

Key Words folate, homocysteine, alcoholics

The liver plays a crucial role in the metabolism of homocysteine; therefore the metabolism of homocysteine in the presence of liver disease may be altered (1, 2). However, there is no information about plasma homocysteine and folate levels in alcoholic patients in relation to hepatocellular injury. The aim of the present study was to investigate the relationship between levels of serum folate and homocysteine in chronic alcoholics, taking into consideration the liver cell injury evaluated by the cytoplasmic and membrane-bound enzyme activity.

The experimental group comprised 80 consecutive chronic alcoholic men aged 23–77 y (mean: 43 y), who were diagnosed with alcohol dependence according to the International Classification of Diseases (ICD-10), category F-10. All patients were interviewed regarding their history of alcohol abuse and classified according to clinical and biochemical data. The control group of 40 healthy men (range: 24–65; mean: 40 y) was also studied. All subjects gave their consent to participate in the studies. The study was approved by the local bioethics committee.

The blood samples were drawn within 12 h after admission. Tubes were immediately centrifuged at $1,500 \times g$ for 10 min at 4°C in order to eliminate the homocysteine efflux from red blood cells to serum and then sera were stored at –87°C until they were analyzed. The samples for folate evaluation were stored in a refrigerator for no longer than 1 mo. Total homocysteine and folate concentrations were measured using the chemiluminescent microparticle immunoassay

technology (CMIA) (Homocysteine, Abbott, Wiesbaden, Germany; Folate, Abbott Ireland, Longford, Ireland) adapted to the Architect i2000 analyzer (Abbott, USA). Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ -glutamyltransferase (GGT) were determined following standard procedures at the Department of Laboratory Diagnostics of University Hospital in Białystok. The distribution of all variables was tested using the Shapiro-Wilk test. Because all variables were not normally distributed ($p < 0.001$ for all tests), the results are presented as median and ranges, and nonparametric statistics were also performed.

The occurrence of hyperhomocysteinemia in alcoholics oscillates in the range between 17 and 30% (3, 4) but the occurrence of folate deficiency fluctuates between 11 and 87% (5–7). The comparison of these values may suggest that other factors, besides folate status, might also play a role in hyperhomocysteinemia in chronic alcohol abuse. One of them is the presence of liver disease (1, 2, 6). The prevalence of low serum folate levels in our study was 42.5%, and high homocysteine concentrations, 60%. Our results showed that folate and homocysteine concentrations in alcoholics did not differ between subjects with normal and high enzyme activity, although the mean folate levels were significantly decreased while homocysteine concentrations were significantly increased in alcoholics (Table 1). On the other hand, the levels of homocysteine in alcoholics with low folate levels were higher than in the subjects with normal folate concentrations.

Many studies showed an inverse correlation between serum concentration of homocysteine and folate (8). In our study there was no correlation between these

E-mail: cylwikb@umwb.edu.pl

Table 1. Serum folate and homocysteine levels in alcoholics.

Group	Folate (nmol/L)		Homocysteine ($\mu\text{mol/L}$)	
	Median	Range	Median	Range
Healthy controls	17.9	9.7–42.4	10.58	7.58–14.96
Total alcoholic group	12.9	4.7–35.1	16.85	8.80–68.35
Alcoholics with normal liver enzymes	12.5	4.7–34.9	16.40	8.80–68.35
Alcoholics with elevated liver enzymes	14.0	5.0–35.1	17.89	10.92–48.95
Alcoholics with normal homocysteine	15.3	5.0–34.9	—	—
Alcoholics with elevated homocysteine	11.3	4.8–35.1	—	—
Alcoholics with normal folate	—	—	15.63	8.80–40.82
Alcoholics with low folate	—	—	17.89	9.11–68.35

The differences between alcoholic subgroups and between alcoholics and controls estimated by Mann-Whitney *U*-test. p^a , between supplementary alcoholic subgroups (i.e. alcoholics with normal homocysteine vs alcoholics with elevated homocysteine), p^c , alcoholics vs controls.

Normal liver enzymes: ALT and AST in the range 5–50 U/L, and GGT 10–75 U/L, elevated liver enzymes: ALT and AST higher than 50 U/L, and GGT than 75 U/L. Normal homocysteine: in the range 5.46–16.20 $\mu\text{mol/L}$, elevated homocysteine: above 16.20 $\mu\text{mol/L}$. Normal folate: in the range 10.92–43.03 nmol/L, low folate: below 10.92 nmol/L.

Table 2. Relationship between plasma homocysteine and folate.

Group	Spearman rank correlation coefficient (R)	<i>p</i>
Healthy controls	−0.442	0.014*
Total alcoholic group	−0.175	0.120
Alcoholics with normal liver enzymes	−0.340	0.014*
Alcoholics with elevated liver enzymes	0.135	0.484
Alcoholics with normal homocysteine	0.191	0.294
Alcoholics with elevated homocysteine	−0.056	0.703
Alcoholics with normal folate	−0.129	0.391
Alcoholics with low folate	0.019	0.914

* Statistically significant correlation.

metabolites, although the co-existence of low folate and high homocysteine concentrations was about 31% (Table 2). The significant negative correlation of folate with homocysteine levels was observed in the control group. This result remains in accordance with data presented by other authors (2). Therefore, we suggest that the association between folate and homocysteine exhibited in the non-abusing healthy population disappears in chronic alcohol abusers in the presence of hepatocellular injury. The confirmation for this speculation is the

existence of a significant correlation between these metabolites in alcoholics with normal liver enzyme activity. The presence of liver disease and the degree of liver damage may influence serum concentrations of folate and homocysteine and mutual association between these metabolites (1, 6). The plasma level of folate is more reduced and plasma homocysteine concentration is significantly higher in non-abstaining alcoholic cirrhosis than in non-alcoholic cirrhosis and abstaining cirrhosis (2). Higher homocysteine concentrations are attributed to the effect of active alcohol drinking.

Although in chronic alcohol abuse the concentrations of folate were mildly decreased and those of homocysteine strongly increased, there was no correlation between these metabolites. The relationship exists only in those subjects that show a normal activity of liver enzymes. Thus, before hepatocellular injury due to alcohol abuse, the correlation in the alcoholic patients exists as in the healthy controls.

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