Moderate Hyperhomocysteinemia in Patients with Huntington's Disease

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Abstract

Hyperhomocysteinemia is considered to be an independent risk factor for cardiovascular diseases and atherosclerosis. Also patients with dementia, either of Alzheimer's or of vascular type, may present with elevated homocysteine levels. All these disorders are linked with older age and since hyperhomocysteinemia is also frequent in the healthy elderly, we were interested to determine homocysteine concentrations in a younger population of demented patients suffering from Huntington's disease. In 15 patients with Huntington's disease and in a control group of similar age, serum homocysteine concentrations were measured and changes were compared to concentrations of folate and neopterin, the latter being an indicator of immune activation. A subgroup of patients with Huntington's disease presented with elevated homocysteine concentrations compared to healthy controls of similar age. Moderate hyperhomocysteinemia was related to lower folate concentrations and also to higher neopterin levels which indicate increased immune activation. The correlation of homocysteine with neopterin concentrations points to a possible relationship between the development of hyperhomocysteinemia and immune activation in the patients.

Key words: homocysteine, Huntington's disease, neopterin, dementia

Introduction

The non-protein forming, sulfur-containing amino acid homocysteine has intensively been studied in recent years. Increased homocysteine concentrations can be i) due to genetic defects (i.e. mutations in the genes encoding for the enzymes methioninsynthase or N-5-methylenetetrahydrofolate reductase), ii) due to metabolic dysfunction or iii) due to insufficient availability of vitamins involved in the metabolism, namely folate, B₁₂ and B₆, required for the conversion of homocysteine (6). Elevated homocysteine levels are known as independent risk factor for cardiovascular disease (1,4), and also peripheral arterial disease, venous thrombosis and renal failure are linked to

genetic hyperhomocysteinemia. More recently, elevated plasma homocysteine levels were reported in patients with neurodegenerative disorders like Alzheimer's disease (3,16) or vascular dementia (16) and also in Parkinson's disease (17). All these disorders are merely diagnosed in older-aged patients and since hyperhomocysteinemia is also frequent in the healthy elderly, we were interested to determine homocysteine concentrations in a younger population of demented patients with suffering from Huntington's disease.

In neurodegenerative diseases, markers of immune activation such as neopterin and soluble cytokine receptors were found increased in the peripheral blood and to correlate to disease progression (14,15,28). Besides, a relationship between moderate hyperhomo-

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cysteinemia and increased neopterin concentrations appears to exist (27) pointing to a role of immune activation and oxidative stress to underlie folate depletion and hyperhomocysteinemia.

In this study, we examined homocysteine concentrations in patients suffering from Huntington's disease, and the concentrations were compared to concomitantly measured concentrations of vitamins folate and B₁₂ and of neopterin.

Materials and Methods

Subjects

We investigated a total of 15 patients (9 male, 6 female) suffering from Huntington's disease (diagnosis proven by molecular genetics or autopsy, aged 40.9 ± 12.3 years (disease duration: 2 - 14 years, mean 7.5 years). There were no clinical signs of or laboratory parameters suggesting infection or nephropathy in the subjects investigated. The study was approved by the local ethic committee, and informed consent was obtained from unaffected relatives of patients with Huntington's disease.

Patients were free of continuous neuroleptic treatment for at least one week, drugs were only given occasionally (10 ggt haloperidol; 12.5 mg clonazepam) for sedation.

Measurements

Blood specimens from patients were taken in the morning after a nocturnal fasting period, and blood was centrifuged immediately at 3000 rpm for 2 x 15 min. Thereafter sera were stored at -20°C until measurement.

Total homocysteine was measured by fluorescence polarization immunoassay (Abbott, Vienna, Austria) according to the manufacturers protocol. Total homocysteine is defined as the sum of all homocysteine species including free and protein-bound mono- and dimeric forms (26). The latter contributing two molecules to the homocysteine level which requires a preceding reduction step in sample preparation.

Folate and vitamin B₁₂ concentrations were determined simultaneously by a double-labelled radioimmunoassay (Magic, No Boil, Chirion Diagnostic corporation, MA, USA). The active isotopes were ¹²⁵I for folate and ⁵⁷Co for vitamin B₁₂. Neopterin was determined by ELISA (BRAHMS Diagnostica, Berlin, Germany).

Result were compared to a control group with similar sex and age distribution which has been examined earlier (16) with the same pre-analytical treatment of blood specimens.

Statistics

Because distribution of data was not normal, statistical analyses were performed by non-parametric Spearman rank correlation and by X²-test (statistical software package SPSS for Windows Version 10.0).

Results

In the patients with Huntington's disease, the median concentrations (interquartile ranges in brackets) were for homocysteine: $9.5 \mu\text{mol/l}$ (7.8 - 15.3), folate: $9.5 \mu\text{g/l}$ (4.20 - 20.9), and vitamin B₁₂: 343 ng/l (277 - 531). Median neopterin concentration was $6.5 \mu\text{g/l}$ (5.2 - 11.7). For homocysteine, the normal range is defined as 5 - 15 $\mu\text{mol/l}$ (13) which agrees well with the concentrations found in our earlier investigation of controls (below 15 $\mu\text{mol/l}$ homocysteine for males and 12 for females (16)) and all controls in this study presented with homocysteine concentrations within these ranges. Compared to this limit, 5 of the 15 patients (33%; $X^2 = 4.84$, $p = 0.03$, X²-test) presented with increased homocysteine concentrations. Compared to the lower limit (5th percentiles) of serum vitamin concentrations in normal subjects (11 $\mu\text{g/l}$ for folate and 185 ng/l for vitamin B₁₂) none of the patients had decreased folate concentrations, and 2 patients (13%) presented with decreased vitamin B₁₂ concentrations. Four patients (27%) had increased neopterin concentrations compared to the 95th percentile of healthy controls = 8.7 nmol/l (8).

An inverse association was found between homocysteine and folate concentrations, and a comparably strong correlation existed between neopterin and homocysteine concentrations (Table I). There was also a significant correlation between age and neopterin concentrations. No correlations were found between duration of the disease and any of the parameters investigated.

Discussion

Moderate hyperhomocysteinemia was found in a subgroup of patients suffering from Huntington's disease, and homocysteine concentrations correlated inversely with folate levels. From the data we conclude that also in Huntington's disease, folate deficiency may be responsible for increased serum homocysteine concentrations. No such correlation existed between homocysteine and vitamin B₁₂. However, the number of patients available may be too low to exclude such a relationship. There was no association of homocysteine concentrations with age or duration of disease, but homocysteine correlated strongly with neopterin concentrations, a marker of immune activation.

Table 1. Spearman rank correlation coefficients (p-values in brackets) between homocysteine, folate, vitamin B12, neopterin and age in 15 patients with Huntington's disease; n.s. = not significant

	Folate	Vitamin B12	Neopterin	Age
Homocysteine	-0.735 (<0.01)	-0.239 n.s.	0.645 (<0.01)	0.320 n.s.
Folate		0.201 n.s.	-0.354 n.s.	-0.040 n.s.
Vitamin B12			0.190 n.s.	0.311 n.s.
Neopterin				0.637 (<0.01)

Since homocysteinuria has been described (9) it became more and more evident that a lack of essential nutritional components like folate or vitamins B₆ and B₁₂ (cobalamin) or the malfunction of enzymes involved in the homocysteine metabolism contributes markedly to the development of hyperhomocysteinemia. When folate and/or vitamins B₁₂ availability is reduced, conversion of homocysteine is limited and hyperhomocysteinemia is likely to develop. It is also assumed that inadequate intake of essential cofactors like folate or vitamins B₆ and B₁₂ may cause accumulation of homocysteine and hyperhomocysteinemia can be at least partly corrected with dietary supplementation of folate or vitamin B₁₂ (11, 23). Nutritional status of severely demented patients very often is subnormal. But independent from the background of hyperhomocysteinemia, supplementation with folate may also be able to correct it also in patients with Huntington's disease.

Huntingtin was found to interfere with cystathionine- β -synthase (2), an L-serine hydrolase adding homocysteine, and is thus probably able to interact with homocysteine metabolism. On the other hand in the present study, homocysteine concentrations correlated with neopterin concentrations. Neopterin is a product of monocyte-derived macrophages activated by interferon- γ , and neopterin concentrations allow to monitor cell-mediated = Th-1 type immune response in vitro and in vivo (7). Elevated neopterin concentrations in cerebrospinal fluid and blood of patients with neurodegenerative disorders like Alzheimer's disease and Huntington's disease, were found earlier to correlate with other markers of immune activation such as soluble TNF receptors and with cognitive abilities of patients (14,15). Since neopterin production coincides with the formation of toxic products like reactive oxygen species during cellular immune response,

increased neopterin concentrations also serve as an estimate of oxidative stress elicited during episodes of immune activation (16). An association exists between higher neopterin concentrations and decreased vitamin E (α -tocopherol) concentrations in nonagenarians and in patients with dementia (22,24).

Oxidative stress appears to play an important role in the loss of neurons characteristic for neurodegenerative diseases (12), and it has even been claimed that homocysteine production is capable of contributing to oxidative processes (19). The correlation found between neopterin and homocysteine concentrations points to an association between immune system activation, oxidative stress and the development of hyperhomocysteinemia. Only enzymatically reduced 5,6,7,8-tetrahydrofolate, the precursor of methyltetrahydrofolate, is the active folate derivative required for the conversion of homocysteine. Tetrahydrofolates are very susceptible to irreversible oxidation because of degradation and is therefore rapidly destroyed in an oxidative milieu (27). Therefore, folate deficiency may develop in patients under oxidative stress conditions even when dietary supply is within the recommended range. Similar relationships were suggested recently to exist for vitamin B₁₂ deficiency in patients with Alzheimer's disease (28).

Our study provides only correlational evidence which cannot confirm a cause-effect relationship. However, similar association between neopterin and homocysteine concentrations has been identified earlier also in other disease states, e.g. in pre-eclampsia and in patients with disturbed glucose metabolism (10,20) and also in Parkinson's disease (29) and in healthy controls (25). Finally a correlation was found between cytokine TNF- α and homocysteine concentrations in patients with atherosclerosis, seropositive for *Chlamydia pneumoniae*, which also supports the

notion of an association between immune activation and the accumulation of homocysteine (30).

There were no signs of infection or inflammation in our patients. Thus, the underlying stimulus of immune activation is unclear, but cellular immune activation appears to coincide with hyperhomocysteinemia. Probably the same stimuli which are responsible for oxidative stress and neurodegeneration are involved to trigger the immune system.

In our patients, age correlated with neopterin concentrations. Higher homocysteine and also neopterin concentrations were shown earlier in older aged people as compared to the young (5,21). Correlation found between neopterin levels and age may be related to a longer disease duration in older patients. Statistics did not reveal such a relationship, but again the number of cases may be too low to exclude such an association. Further studies will be necessary to clarify this point.

We conclude that a subgroup of patients with Huntington's disease presents with moderate hyperhomocysteinemia which coincides with cellular immune activation. Oxidative stress could be involved to destroy cofactors necessary for the conversion of homocysteine. Dietary vitamin supplementation may help to overcome possible consequences of enhanced requirement of antioxidant vitamins like folate.

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