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SHORT COMMUNICATION

Pseudodeficiency of glutamine in infant liver disease

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Abstract γ-Glutamyltransferase (γ-GT) is an early marker for cholestasis and has the capability of glutamine-deamidation. Two infants with elevated serum γ-GT had a decreased serum glutamine. A time course of glutamine and glutamate concentration changes was performed. This revealed a time dependent decrease of glutamine far below the normal lower limit while glutamate increased above the normal upper limit. In conclusion, increased in vitro γ-GT can cause pseudodeficiency of glutamine. To avoid pitfalls, physicians should inform the laboratory on accompanying pathologies.

Keywords γ-Glutamyltransferase · γ-GT · Glutamine deficiency · Cholestasis

Introduction

The enzyme γ-glutamyltransferase (γ-GT, *EC 2.3.2.2*) is a membrane-bound heterodimeric glycoprotein located on the luminal membrane of biliary and many other cells. γ-GT is responsible for the extracellular catabolism of glutathione, the main thiol intracellular antioxidant agent in cells, and catalyzes the transfer of the γ-glutamyl moiety of glutathione to various peptide acceptors (Taniguchi and Ikeda 1998; Whitfield 2001). In general, it is an early laboratory marker for liver cell damage, alcohol misuse and cholestasis. Increased concentrations of γ-GT are found in most cholestatic disorders when bile salts act with γ-GT and lead to an increased canalicular secretion (Colombo et al. 2000). If there is a defect in the synthesis of bile acids or a defect of bile secretion, such as in progressive familial intrahepatic cholestasis (PFIC) disorders, γ-GT will be normal or low despite the presence of intrahepatic cholestasis (Colombo et al. 2000). However, in practice, γ-GT can be regarded as a sensitive marker of cholestatic liver disease.

In addition, γ-GT has an effective glutamine-deamidating activity leading to a decrease of glutamine and an increase of glutamate and ammonia in a blood sample (da Fonseca-Wollheim 1990). The aim of this study is to call the attention to this phenomenon and to help avoiding possible pitfalls of amino acid analysis.

Materials and methods

Subject of the study are two 6-weeks-old male infants who both presented with laboratory signs of liver disease. Patient 1 showed a direct hyperbilirubinemia on day 2 of life that was interpreted in the absence of any further pathological finding as idiopathic neonatal hepatitis. Maximum serum level of γ-GT in this patient was 1105 U/l (normal upper limit for age 230 U/l) which was documented on the day of the first amino acid analysis. Patient 2 was treated for a bacterial bronchopneumonia and developed signs of a hepatopathy on a routine control on day 12. The cause of the liver cell damage was thought to be toxic since no other diagnosis could be made. Five days prior to the first amino acids analysis in patient 2, γ-GT reached its maximum serum level of 4,287 U/l. In both patients, all signs of liver disease resolved spontaneously with a normalization of the γ-GT levels within few months.

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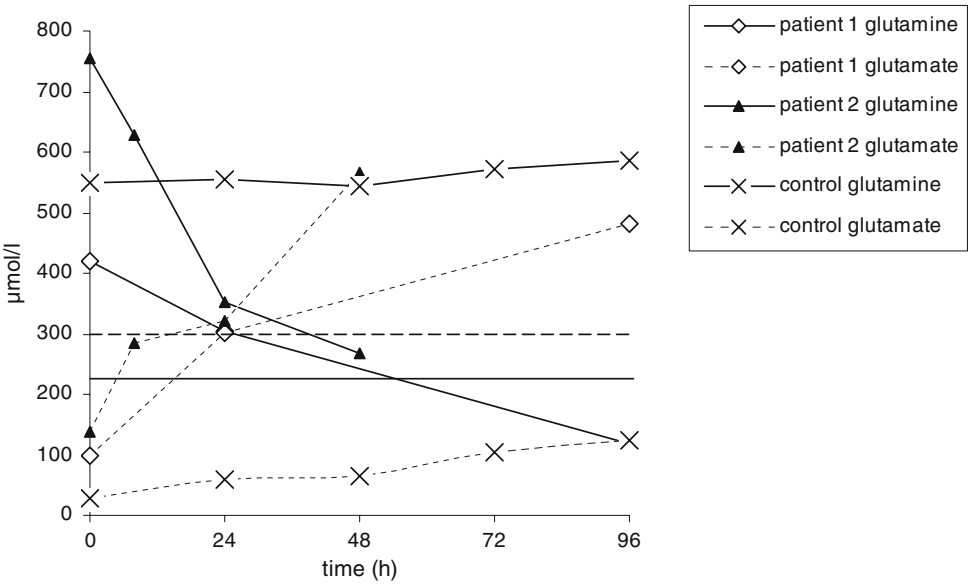

Fig. 1 Diagram showing the time dependent changes of the glutamine and glutamate concentrations in the samples of both patients and of the control, respectively. The *dashed line* indicates the normal lower limit of the serum glutamine level. The *straight line* indicates the normal upper limit of the serum glutamate level

During the metabolic work-up in both patients, low levels of serum glutamine (105 and 31 µmol/l, respectively, normal range 300–800) with elevated levels of serum glutamate (614 and 398 µmol/l, respectively, normal range 70–220) were found using a quantitative analysis of the amino acid concentration by a standard cation exchange chromatography on a LC 3001 amino acid analyzer (Eppendorf-Biotronic, Hamburg, Germany). On the basis of the known glutamine deamidating activity of γ-GT, blood sampling was repeated and the following precautions were taken: samples were centrifuged immediately, treated with 200 µl 3% sulfosalicylic acid to remove proteins and then send to the laboratory. Measurements of amino acids were done immediately in both patients, after 24 and 96 h in patient 1, and after 8, 24, and 48 h in patient 2, respectively. In addition, a control serum sample (γ-GT level of 6 U/l) was investigated after the identical precautions.

The parents of both patients and of the control proband gave their informed consent into the above investigations.

Results

In both patients, initial concentrations of glutamine were far below the normal lower limit but based on the normal neurological presentation a primary glutamine deficiency was unlikely and a secondary glutamine deficiency had to be assumed. Knowing the grossly elevated γ-GT, deamidation of glutamine as the cause was hypothesized and precautions met in order to avoid this effect. When control blood samples were drawn and treated as described above, the first measurements revealed normal glutamine concentrations of 420 and 756 µmol/l, respectively. Likewise, the levels of glutamate were within the reference range (100 and 139 µmol/l, respectively). When these samples were repeatedly investigated while stored at room temperature, levels of glutamine dropped to 117 µmol/l in patient 1 and to 268 µmol/l in patient 2 with the levels of glutamate rising well above the normal upper limit. There were no relevant changes in the respective concentrations in the control sample. The time dependent courses of glutamine and glutamate are shown in Fig. 1.

Discussion

When profiles of amino acids in plasma are interpreted, attention is usually paid to high levels of amino acids such as in phenylketonuria and tyrosinemias. Also, newborn screening for metabolic disorders is based on elevations and not on a decrease of amino acids (Fearing and Marsden 2003).

There are a few well known pitfalls in this respect: hemolysis in the sample can lead, via the action of arginase from red blood cells, to artificial decreases of arginine which is then converted into ornithine (Scriver et al. 1971). Also, delays in sample preparation can result in low cysteine levels by binding to protein in the hemolysate (Scriver et al. 1971; Schaefer et al. 1987).

In addition, there are a small number of disorders that affect the biosynthesis of amino acids and that are accompanied by low levels of amino acids. Examples of this are disorders of proline and serine metabolism. In Δ1-pyrroline-5-carboxylate synthase deficiency, low levels of proline, ornithine, arginine and citrulline can result (Baumgartner et al. 2000). In 3-phosphoglycerate dehydrogenase deficiency and in phosphoserine phosphatase deficiency, decreased levels of serine can be found (Jaeken

et al. 1996). Another example is the deficiency of glutamine synthetase, a rare disorder that presents with a pronounced decrease of glutamine levels in plasma, urine, and cerebrospinal fluid (Häberle et al. 2005).

In the patients described here, a secondary glutamine deficiency had to be expected since primary glutamine synthetase deficiency was unlikely given the normal neurological presentation. On the basis of the known liver disease in the two patients, a pseudodeficiency of glutamine caused by the action of γ-GT was assumed. This could be confirmed by the striking time dependent decrease of the glutamine concentration in samples from both patients after 96 and 48 h, respectively. During the same time, glutamate concentrations increased from normal to markedly elevated levels.

With respect to the known glutamine-deamidating activity of γ-GT there are two ways to avoid a possible pitfall. One would be to inhibit the deamidation of glutamine in the sample by adding 2 mM borate plus 5 mM L-serine (da Fonseca-Wollheim 1990). The other, more practicable way is the immediate processing of the sample as performed in the above patients. Most importantly, however, are a close collaboration of physicians and the laboratory ensuring the transfer of relevant information on the patient allowing for an accurate interpretation of amino acid profiles including both the elevated but also the decreased concentrations.

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