

Increased Serum Concentrations of Carbohydrate-Deficient Transferrin (CDT) in Patients with Cystic Fibrosis

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ABSTRACT

Carbohydrate-deficient transferrin (CDT) has been reported to be one of the best laboratory markers in serum (S) for detection of alcohol abuse.

We have studied S-CDT values in cystic fibrosis (CF) patients and show that CF patients have increased S-CDT values without high alcohol consumption. CF patients have abnormalities in their protein glycosylation and sialylation, which may explain the increased S-CDT values.

INTRODUCTION

Chronic high intake of alcohol results in damage to many different organs including development of chronic pancreatitis, liver cirrhosis and myopathy. Alcoholics are overrepresented among patients in the hospitals and several biochemical markers have been developed to diagnose alcoholic abuse (6,17).

Carbohydrate-deficient transferrin (CDT) has been used for more than a decade as a marker in serum for alcoholic abuse (11). Transferrin (T) has several isoforms that differ in the degree of sialylation of the two N-linked oligosaccharide chains (8). The least sialylated isoforms of T, with 0 (asialo T) and 2 (disialo T) sialic acids are usually referred to as S-CDT. The formation of S-CDT in individuals with high alcohol consumption is believed to be due to inactivation of enzymes in the Golgi apparatus (3). Pathological S-CDT values have also been reported in patients with carbohydrate-deficient glycoprotein syndrome and galactosaemia (13).

Cystic fibrosis (CF) is the most common fatal genetic disease in the United States today (9). It occurs in approximately one of every 3,300 births and affects approximately 30,000 children and young adults. One in twenty-nine Americans is a carrier of the gene. In Sweden there are approximately 475 individuals with CF. There are more than 600 different known mutations that cause CF. This makes it sometimes difficult to diagnose CF by DNA technology. The sweat test is

currently the standard diagnostic test (15). This test measures the amount of salt in the sweat and a high level of salt indicates that a person has CF. Sweat tests are time consuming and thus costly and individuals with a high alcohol consumption may also have positive test results. Previous studies have shown that CF patients have abnormalities in their glycosylation (5), sulfatation (2) and sialylation (4) of membrane and plasma proteins. Such changes may interfere with the measurement of S-CDT. We have used high-performance liquid chromatography to quantify the different isoforms of transferrin in healthy controls and patients with cystic fibrosis. The purpose of the study was to evaluate if CF may cause an increase in S-CDT and to see if isoforms of Tf could be used to diagnose CF.

MATERIALS AND METHODS

Patients

Twenty CF patients, aged 7 to 37 years were studied. CF diagnosis was established on the basis of typical clinical symptoms and abnormal sweat tests. None of the patients had hepatic disorders.

Blood was collected in serum tubes without additive (367609, Becton Dickinson, Rutherford, NJ). All patients gave informed consent prior to blood sampling. The serum samples were stored at -20°C until tested.

Analysis of carbohydrate deficient transferrin (S-CDT)

Automated ion-exchange chromatography

Twenty CF patients were analysed. Determination of S-CDT and transferrin isoforms in serum was performed according to Jeppson et al. (7) at the Department of Clinical Chemistry, University Hospital, Uppsala. The reference interval for the method is <1.2% and the total imprecision for the assay is 3.5% at a S-CDT value of 2,4 %. A group of 149 healthy blood donors and medical students were used as controls.

CDTect™

S-CDT was also analysed by CDTect™ (Pharmacia, Uppsala, Sweden) in twelve CF patients. Pharmacia performed the assay.

Statistical analysis

Differences between groups were calculated by Friedman ANOVA. The statistical analysis was performed with Statistica 4.5 (Statsoft Inc., Tulsa, OK). $P < 0.05$ was considered significant.

RESULTS

Transferrin isoforms in serum measured by ion-exchange chromatography

The distribution of the different serum isoforms in healthy individuals was: disialo T 0.735%±0.238 (mean±S.D.), trisialo T 4.575%±1.315, tetrasialo T 84.886%±1.717, pentasialo T 9.794%±1.760.

No asialo T was detected in any of the healthy controls.

The mean serum values for the different isoforms in CF patients were: disialo T 0.954% (p<.04), trisialo T 2.584% (p<.0004), tetrasialo T 84.118% (p=.65), pentasialo T 12.347% (p<.03). No asialo T was detected in any of the CF patients.

CDTect™

Six male and six female CF patients were analysed. The mean serum values were 18.5 U/L for men and 18.3 U/L for females. Two of the boys had serum CDTect™ values above 20 U/L. These two boys were seven and eight years old and came from two different families. Serum values for healthy controls were < 20 U/L for men and < 26 U/L for women according to the laboratory at Pharmacia.

DISCUSSION

Transferrin is present in high concentrations in serum. It is a glycoprotein with two bi- or tri-antennary carbohydrate chains, each terminated with two or three sialic acids (8). Differences in the number of carbohydrate chains and sialic acids will alter the isoelectric point of the transferrin molecule. The isoforms related to alcohol abuse contain less sialic acids and have a higher isoelectric point than other isoforms of transferrin. Ion exchange chromatography separates serum transferrin into isoforms depending on sialic acid content, iron saturation and amino acid content. After saturation of the transferrin molecule with iron, S-transferrin is normally separated into four isoforms according to their isoelectric point (pI): pI 5.2 (5 sialic acids), pI 5.4 (4 sialic acids), pI 5.6 (3 sialic acids) and pI 5.7 (2 sialic acids). High alcohol consumption will result in an increase of the pI 5.7 fraction and an additional pI 5.9 (0 sialic acids) fraction may appear. The fractions with pI 5.7 and 5.9 are called carbohydrate-deficient transferrin. Several methods have been used for quantification of S-CDT: HPLC (7), isoelectric focusing/western blotting (16), anion-exchange chromatography followed by RIA (12) or nephelometry (10).

S-CDT has a sensitivity of 82% and a specificity of 97% for alcohol abuse (11) in selected materials. The sensitivity of S-CDT varies between different studies depending on the examined population, but the specificity of S-CDT is usually high. False-positive results have previously been

reported in patients with primary biliary cirrhosis, genetic variants of transferrin, carbohydrate-deficient glycoprotein syndrome or galactosaemia (13).

The formation of S-CDT in individuals with high alcohol consumption is believed to be due to inactivation of enzymes in the Golgi apparatus (3). In CF cells there is a defective acidification of the trans-Golgi/trans-Golgi network, of prelysosomes and of endosomes as a result of diminished Cl⁻ conductance (1). This results in a reduced sialylation of proteins and lipids. These abnormalities can result from defective acidification because vacuolar pH regulates glycoprotein processing. Thus, both patients with high alcohol consumption and CF patients have a defective sialylation that may result in increased S-CDT levels.

We have studied the levels of S-CDT in serum from patients with CF. CF is the most common life-shortening genetic disorder in populations of European origin. The basic defect in CF is a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome seven (14), which results in a faulty transport of sodium and chloride and causes the body to produce abnormal exocrine secretion. Symptoms come from many organs, especially the airways and the gastrointestinal tract, but also from liver, sweat glands and the reproductive system. It is sometimes difficult to diagnose CF. A simple inexpensive serum assay such as S-CDT could potentially be used as a screening assay for CF.

We originally used the CDTECTTM method. The CDTECTTM method measures the absolute amount of S-CDT and not S-CDT as a percentage of total transferrin. A patient with increased serum transferrin values due to iron deficiency will therefore have a higher CDTECTTM value. CF patients may have an iron deficiency. We thus changed method from CDTECTTM to ion-exchange chromatography, which also gave significantly elevated serum levels of CDT in CF patients in relation to healthy controls. Thus, we have found increased S-CDT levels in CF patients with two different methods. The method will probably influence the results as we found subnormal values of trisialo T in our CF patients and some S-CDT methods also measure trisialo T.

All our CF patients denied high alcohol consumption, had no increase of any other serum marker of alcohol abuse (e.g. S-ALAT, S-γ-GT, MCV) and had no history of severe liver disease or other diseases than CF (and CF related disorders). Some of the patients were below 10 years of age, which makes high alcohol consumption unlikely.

Conclusion: Cystic fibrosis patients have increased S-CDT levels due to defective sialylation and not due to high alcohol consumption. CF patients also have decreased serum levels of trisialo T. Further studies have to be performed to evaluate if S-CDT could be used as a screening assay for CF.

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