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Capillary zone electrophoresis of transferrin and EDTA samples in congenital disorders of glycosylation screening: CaNOt do, really?

Congenital disorders of glycosylation (CDG) are a rapidly expanding family of inborn errors of metabolism affecting protein glycosylation pathways. Diagnosis is difficult since there is most often no specific clinical sign, and therefore involves additional biochemical and genetic investigations. The first developed biochemical test in CDG screening was transferrin (Trf) isoelectric focusing [1]. Nowadays, a commonly used technique is capillary zone electrophoresis (CZE), which offers benefits such as accurate quantification of Trf glycoforms, increased analysis speed and ability to process multiple samples [2]. In healthy subjects, Trf is mostly tetrasialylated (4-sialoTrf; ~80% of total Trf glycoforms) with low levels of 3-sialoTrf (<6%) and 2-sialoTrf (<1.3%). In CDG, N-linked glycosylation is usually affected, with a loss of terminal sialic acids (SA) residues resulting in variably increased proportions of 3-sialo to 0-sialoTrf at the expense of 4-sialoTrf.

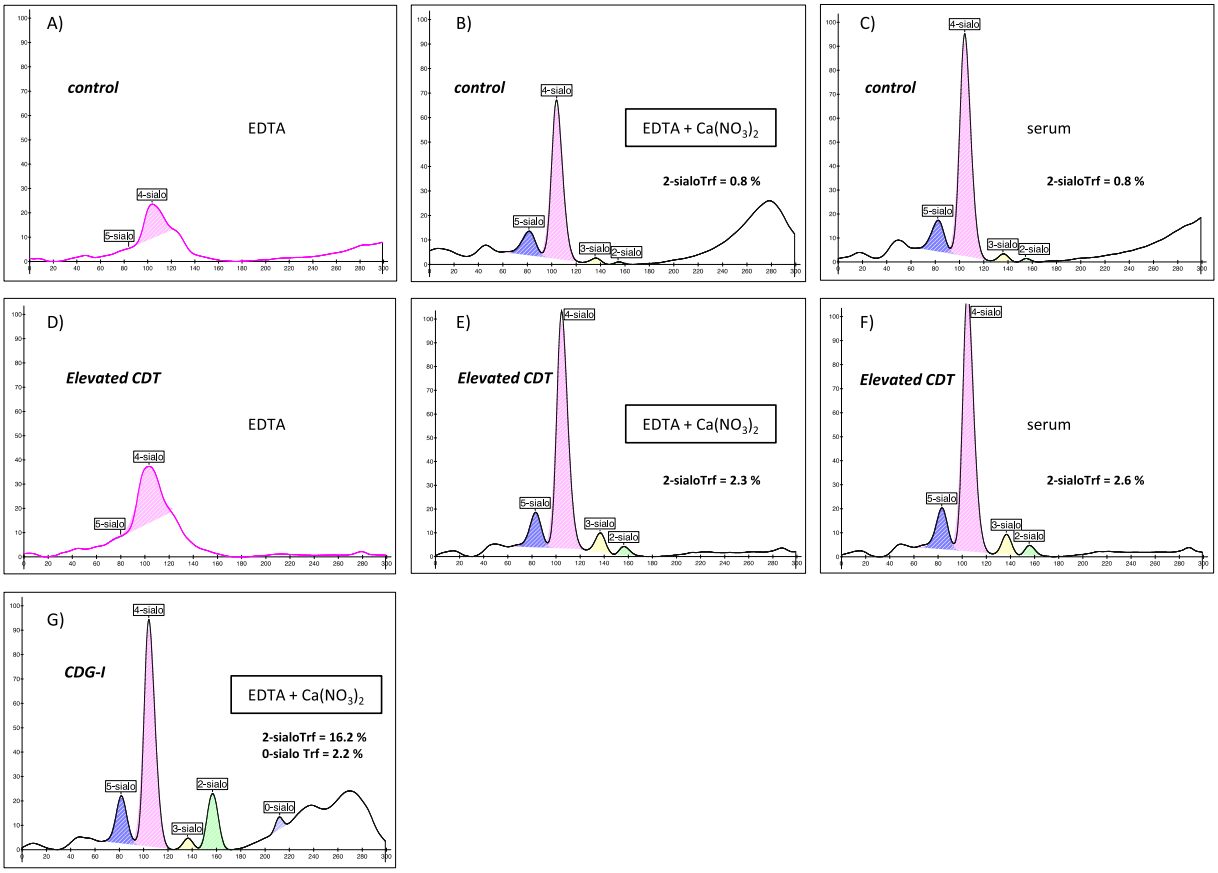

Fig. 1. Trf CZE profiles from serum, $Ca(NO_3)_2$-treated and untreated EDTA samples, processed by the Sebia Capillarys 2 analyser. In (A), analysis of an EDTA sample from a healthy individual results in an uninterpretable profile. $Ca(NO_3)_2$ treatment of the EDTA sample recovers the profile (B), which is highly similar to the profile obtained from corresponding serum sample (C). This is also shown for a patient known for chronic alcohol abuse with elevated CDT, with the uninterpretable EDTA sample in (D), the treated EDTA sample in (E) and the corresponding serum sample in (F). In (G), the profile obtained from a $Ca(NO_3)_2$-treated EDTA sample from a DPAGT1-CDG patient displays a typical Type I CDG pattern (CDG-I).

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In our laboratory, Trf CZE for CDG screening is performed on a Capillarys 2 Flex Piercing analyzer (Sebia, France) using the Capillarys CDT kit, originally developed for alcohol abuse detection and follow-up [3]. Briefly, samples are loaded onto the analyzer and mixed with an alkaline buffer containing iron (Fe^{3+}) before the CZE-based separation of Trf glycoforms. Overloading with Fe^{3+} is indeed required to ensure that Trf glycoforms are strictly separated according to their number of negatively charged terminal SA.

A major problem that emerged after implementing this technique was its incapacity to process EDTA plasma samples frequently sent to our laboratory. Unlike with serum or heparin plasma samples, we were not able to obtain interpretable profiles (Fig. 1A and D). EDTA, a cation chelator, would bind Fe^{3+} in the CZE reagent, preventing full Trf saturation. Since most samples come from young children, it proves difficult to ask clinicians for new ones collected in the right tubes. Therefore, to ensure blood conservation and thus patient comfort, we developed a simple technique to allow Trf CZE analysis of EDTA plasma samples.

We tried preventing reagent Fe^{3+} chelation by EDTA by adding 1/20^{th} (v/v) of 0.5 M calcium nitrate $Ca(NO_3)_2$ aqueous solution to the EDTA plasma sample before CZE. The idea was that Ca^{2+} added would fully bind EDTA molecules so that Fe^{3+} would be free to overload Trf as usual. This was first successfully tried out in various EDTA samples from healthy subjects (n = 18) as illustrated in Fig. 1B and C. The determined means of differences (sera minus corresponding treated EDTA samples) and standard deviations (SD) in % values were −0.7 (0.5), +0.6 (0.6), +0.1 (0.2) and 0.0 (0.1) for 5-sialo, 4-sialo, 3-sialo and 2-sialoTrf, respectively. These differences were fully acceptable since they did not change the clinical interpretation of results. We then analyzed EDTA samples (n = 4) from alcohol abusers with pathologically increased hyposialylated Trf. Patterns after $Ca(NO_3)_2$ treatment (e.g., Fig. 1E) were fully interpretable and highly similar to those from corresponding serum samples (Fig. 1F). Here, the determined means of differences were −0.3 (0.6), +0.3 (0.7), 0.0 (0.1) and +0.1 (0.1) for 5-sialo, 4-sialo, 3-sialo and 2-sialo-Trf, respectively. Therefore, as with the control samples tested, these differences were deemed fully acceptable for routine CDG screening. To illustrate this, we analyzed a $Ca(NO_3)_2$-treated EDTA sample from a CDG-affected individual and we found a typical type I Trf pattern (Fig. 1G). Of note, we also evaluated another calcium salt, calcium chloride $CaCl_2$. It precipitated when mixed to the CZE reagent, probably because of its poor solubility under alkaline conditions. As this phenomenon put the capillaries at risk of damage, we discarded it as an alternative to $Ca(NO_3)_2$.

We therefore showed that a frequent and inconvenient pre-analytical problem, EDTA tubes being unusable in Trf CZE, could be solved by the simple addition of aqueous $Ca(NO_3)_2$, allowing optimal CDG screening while avoiding uncomfortable and unneeded additional blood sampling.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

CDG	Congenital disorder(s) of glycosylation
CDT	Carbohydrate deficient transferrin
CZE	Capillary zone electrophoresis
EDTA	Ethylenediaminetetraacetic acid
SA	Sialic acid
Trf	Transferrin

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