Journal of Chromatography, 274 (1983) 337—340
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1623

Note

Studies on multiple molecular forms of transferrin

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(Received November 18th, 1982)

The clinical significance of the iron-transport protein transferrin has been known for more than 30 years. Interpretation of its function and variability has been published [1]. With isoelectric focusing this protein has been found to occur in multiple molecular forms [2]. These forms differ in the glycoside residues attached to the polypeptide and/or in the amount of iron bound to the protein [3]. The presence of seven multiple molecular forms in human serum and nine in cerebrospinal fluid has been demonstrated with immunofixation after isoelectric focusing as described by Stibler [2]. One of these multiple molecular forms, having an isoelectric point (p*I*) of 5.7 ($Tf_{5.7}$), has attained a special interest. The ratio of the $Tf_{5.7}$ concentration and the total transferrin (Tf_{tot}) concentration, i.e. ($Tf_{5.7}/Tf_{tot}$) × 100%, has been proposed to be inversely correlated to hepatocyte membrane function and might be used as a marker of damage of the membrane function such as is caused by alcoholic abuse [3, 4].

In order to study this further we have developed an analytic procedure using isoelectric focusing and zone immunoelectrophoretic assay [5] for quantitation of the concentration of $Tf_{5.7}$ and the relation of the $Tf_{5.7}$ concentration to the Tf_{tot} concentration.

MATERIAL AND METHODS

Serum samples were obtained at the Karolinska Hospital, Stockholm, Sweden, from healthy blood donors with ages in the range 21—65 years (39 male, 11 female). The serum samples were frozen within 1 h in small aliquots at −24°C and analyzed within one month. The method used has been presented in ref. 5. In addition, we now incorporate (as a spacer) glycylglycine

0378-4347/83/$03.00

(free base, Sigma, St. Louis, MO, U.S.A.) at 1% (w/v) in the gel, which improves the separation of $Tf_{5.7}$ and the component anodal to this by about 1 mm. This facilitates the isolation of $Tf_{5.7}$.

RESULTS AND DISCUSSION

Absolute concentrations of Tf_{tot} and $Tf_{5.7}$ are summarized in Tables I and II, respectively. The ratio $Tf_{5.7}/Tf_{tot}$ in 50 sera ranged from 2% to 7%. The frequency distribution is shown in Fig. 1. Some additional calculations are given in Table III. Plotting Tf_{tot} against $Tf_{5.7}$ (not shown) displays a regression coefficient (r) of only 0.47.

As can be seen in Table III there appears to be quite a large biological variability, especially for the $Tf_{5.7}$ concentrations. The relative standard deviation (R.S.D.) is 28%. These figures include the method error, which for $Tf_{5.7}$ was typically about 10%. The biological variability is more truly reflected by the R.S.D. of the Tf_{tot} concentration (biological 16%, method 3%). The $Tf_{5.7}/Tf_{tot}$ ratio consequently has an R.S.D. of 24%, including these errors.

TABLE I

THE FREQUENCY DISTRIBUTION OF THE Tf_{tot} CONCENTRATION IN 50 NORMAL SERUM SAMPLES

i	Classes (Tf_{tot}, g/l)	n_i
1	1.75—2.00	2
2	2.00—2.25	11
3	2.25—2.50	8
4	2.50—2.75	3
5	2.75—3.00	9
6	3.00—3.25	12
7	3.25—3.50	1
8	3.50—3.75	3
9	3.75—4.00	1
10	4.00—4.25	1

TABLE II

THE FREQUENCY DISTRIBUTION OF THE ABSOLUTE $Tf_{5.7}$ CONCENTRATION IN 50 NORMAL SERUM SAMPLES

i	Classes ($Tf_{5.7}$, mg/l)	n_i
1	62.5— 75.0	5
2	75.0— 82.5	7
3	82.5—100.0	6
4	100.0—112.5	7
5	112.5—125.0	10
6	125.0—132.5	4
7	132.5—150.0	3
8	150.0—162.5	4
9	162.5—175.0	4
10	175.0—182.5	1

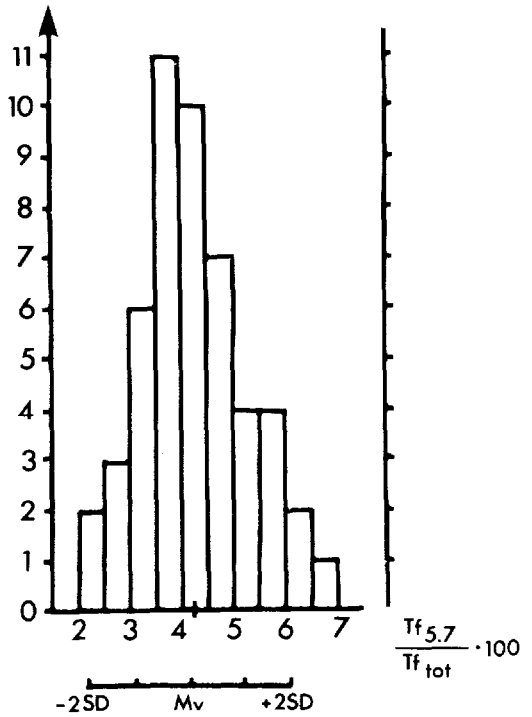

Fig. 1. Histogram presentation of the $Tf_{5.7}$ concentration frequency distribution. The mean value (Mv) and the ± 2S.D. range are also indicated. The denomination is per cent and the division is 0.5%.

TABLE III

THE MEAN VALUES, ± S.D. AND ± 2S.D. RANGES AND THE RELATIVE STANDARD DEVIATION OF THE PARAMETERS SUMMARIZED IN TABLES I AND II

	Mean (g/l)	Range of mean ± S.D.	Range of mean ± 2S.D.	R.S.D. (%)
Tf_{tot}	2.73	2.21 —3.25	1.67 —3.77	19
$Tf_{5.7}$	0.11	0.080—0.140	0.650—0.170	28
$Tf_{5.7}/Tf_{tot}$%	4.2	3.2 —5.2	2.2 —6.2	24

Some controversy is introduced as there seems to be no direct correlation between $Tf_{5.7}$ and Tf_{tot}. Probably, $Tf_{5.7}$ has lost some of its capping sialic acid residues [3] and thus should be cleared from the blood with a certain normal capacity per time unit. The proposed usage of the $Tf_{5.7}/Tf_{tot}$ ratio as an indicator of alcohol abuse supposes a change in one of these parameters, i.e. $Tf_{5.7}$ [3]. Damaged hepatocytes are supposed to have a reduced capacity for removal of this transferrin species, thus increasing the $Tf_{5.7}/Tf_{tot}$ ratio. In normal sera a high concentration of Tf_{tot} should therefore be accompanied by a high $Tf_{5.7}$ concentration, thereby not significantly altering the ratio between synthesis and removal. The possibility to detect damage in this system required a correlation between these parameters. Normally, a correlation coefficient greater than 0.5 is said to validate correlation. We are quite surprised at the low

correlation obtained (0.47). The understanding of the $Tf_{5.7}/Tf_{tot}$ ratio as a marker of hepatic (mal-)function requires additional studies. The 50 values for the $Tf_{5.7}/Tf_{tot}$ ratio is published to serve as a reference for such purposes.

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