Studies of transferrin in serum of workers exposed to organic solvents

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ABSTRACT Earlier studies have shown that determination of the serum concentration of different forms of transferrin (isotransferrins) may be used to detect hepatic effects caused by alcohol abuse. The isotransferrin variant with an isoelectric point of 5.7 has been compared with the total amount of serum transferrin in order to study hepatic effects caused by occupational exposure to organic solvents. Eighteen workers from a paint industry were tested before and after their holiday and compared with two different groups, a total of 60 subjects. The solvent workers had significantly higher values both before and after their holiday. This suggests that the effects on the liver caused by organic solvents are similar to those caused by alcohol abuse and that this is a long term effect. The level of exposure to organic solvents was below the Swedish threshold limit values.

Serum transferrin is a protein synthesised and catabolised in the liver; its main function is iron transportation in the blood. Transferrin has many variants (isoproteins) which may be separated by isoelectric focusing (IEF). Except for differences that are genetically caused (differences in the amino acid sequence) the main difference in isoelectric point (pI) between the variants is due to the variable number of sialic acid groups attached to the carbohydrate parts of the transferrin molecule.^{1,2} One of these variants, with a pI of 5.7 (Tf_{5.7}), has been shown to be increased after alcohol abuse.^{1,3} In the present study a similar effect is shown to follow occupational exposure to organic solvents.

Subjects and methods

Workers exposed for between one and 10 years in a paint industry plant were studied. Blood sera were obtained on two different occasions; sample A was taken in the middle of an ordinary working week and sample B on the first or second workday after holiday (the latter being at least two but usually three or four weeks). All samples were frozen at -70°C within one day and were analysed within one month. The referents were blood donors (referents D) and a group of non-exposed subjects (referents M) working in the same area as the exposed workers. Samples from the

referents and exposed workers were treated and analysed in the same way. None of the workers or the referents reported any alcoholic problems. Table 1 shows the composition of the groups. Interviews using a standardised form, about drug and alcohol usage were recorded as confidential and codified information for the exposed and referents M. Information obtained about alcohol usage a few weeks before the blood sampling was regarded as reliable due to good personal contact and cooperation between the workers and the interviewing nurse. Only a few used drugs at the time of the study; two used oral contraceptives and one was taking antibiotics.

Transferrin measurements were made as described earlier.⁴ After iron saturation, the transferrin with pI 5.7 (Tf_{5.7}) was separated by isoelectric focusing and quantified with zone immunoelectrophoresis assay (ZIA). The total concentration of transferrin (Tf_{tot}) was also determined with ZIA. The transferrin ratio (Tf_{ratio}) was calculated as:

$$Tf_{ratio} = Tf_{5.7}/Tf_{tot} \times 100$$

Environmental air sampling for solvents was carried out by pumping a measured volume of air through sampling tubes containing charcoal. All the air samples, obtained during ordinary working operations, were taken for two to four hours in the breathing zone of the workers using personal samplers on typical production days as estimated by the health centre staff and the workers. Extraction of the char-

coal was made with carbon disulphide before analysis by gas chromatography. Wilcoxon's rank test and Wilcoxon's signed rank test were used for statistical evaluations.⁵

Results

The results of the air analysis are presented in table 2. The values were below the Swedish threshold limit values in force when this study was performed (1984).

Table 1 shows the total Tf values and the Tf_{ratio} for the different groups. The total amount of Tf was well within the normal range for all four groups. There

was no difference in the Tf_{ratio} between the two referent groups but with the exposed, a statistically significant increase was found at the 95% probability level (table 3). Owing to the reliability of the alcohol usage records we do not think that this was due to a different use of alcohol. On the other hand, statistical analysis showed no significant difference between the exposed workers on a group basis before and after vacation (table 3). The figure illustrates the difference between referents M and the exposed group before and after the holiday. The individual with the high Tf_{ratio} after holiday admitted a high alcoholic intake during the holiday and up to the day when sample B

Table 1 Age, sex, and transferrin data of subjects. Tf_{tot} is mean value of total serum transferrin. Tf_{ratio} is the quotient between the concentrations of transferrin with isoelectric point 5.7 and Tf_{tot} and expressed as a percentage. Referents D and M are described in the text. Exposed A and B refers to exposed workers sampled before and after the holiday respectively

Group	No	Women	Men	Age range (years)	Tf _{tot} (g/l)	Tf _{ratio}		
						Mean	SD	Range
Referents D	26	13	13	23-65	2.22	1.84	0.84	0.7-4.1
Referents M	17	8	9	23-61	2.31	2.18	0.36	1.6-2.9
Exposed A	14	4	10	20-63	2.24	2.71	0.70	1.7-3.7
Exposed B	12	4	8	27-63	2.06	2.84	0.98	1.6-5.0

Table 2 Results of analysis of air samples taken in the breathing zone of each worker. The hygienic effect is obtained by determining the quotient of the calculated average air concentration and the corresponding threshold limit value for each substance adding all quotients. All values are given in mg/m³

Sample No	White spirit	Xylene	Toluene	Ethylacetate	Ethanol	Butylacetate	Ethylglycol-acetate	Hygienic effect
1	137	47	—	—	—	—	—	0.41
2	247	70	—	—	—	—	—	0.69
3	60	26	—	103	—	13	—	0.36
4	66	20	—	24	—	—	—	0.22
5	179	36	—	—	—	—	—	0.46
6	70	21	—	—	—	—	—	0.20
7	42	51	27	14	10	—	13	0.36
8	49	89	—	—	30	—	19	0.41
9	157	21	—	—	—	—	—	0.37
10	164	24	—	—	—	—	—	0.40
11	69	16	—	—	18	—	—	0.19
12	233	42	—	—	14	—	—	0.60
13	42	16	—	23	—	—	—	0.16
14	25	—	—	—	—	—	—	0.05
15	39	70	—	14	—	—	—	0.30
16	90	192	—	26	—	—	—	0.77
17	43	—	239	32	49	17	—	0.94
18	27	—	34	—	—	—	—	0.16
19	49	22	—	22	—	—	—	0.19
20	135	40	—	55	—	—	—	0.46
21	30	20	—	155	10	—	—	0.34
22	44	22	—	75	615	—	—	0.48
Threshold limit value	500	350	350	700	1900	700	500	

Table 3 Statistical comparison of the groups. The first two values are based on Wilcoxon's rank test and the last on Wilcoxon's signed rank test. A 95% level of significance was used

Groups	Obtained value	Limit value	Conclusion
Referents M v referents D	z = 1.93	z _{0.975} = 1.96	No difference
Exposed A v referents M	z = 2.01	z _{0.975} = 1.96	Difference
Exposed A v exposed B	R = -20.5	5% = 11	No difference

Cumulative relative frequency of differences in Tf_{ratio} (Tf_r) between non-exposed group (M) and exposed workers before (A) and after holiday (B). Single high value in group B is discussed in text.

was taken. The others did not report an appreciably higher alcohol consumption during their holidays than before sample A was taken. The two referent groups did not differ statistically. The group from the health centre (M) has a slightly higher Tf_{ratio} mean value than the blood donor group (D). We cannot explain this by, for example, known differences in alcoholic consumption between the two groups.

Discussion

Raised Tf_{ratio} may indicate that the liver does not function normally. It is not known if this is a primary effect on membrane function or due to secondary cellular influences.

The Tf_{ratio} is believed to be influenced by the liver cell membrane function. Our hypothesis is based on the fact that a high Tf_{ratio} often results in a raised concentration of $Tf_{5.7}$ that has a lowered content of sialic acid compared with the main Tf component. The sialic acid groups of transferrin are believed to be split off the protein molecules in the blood towards the end of their lifetime. Human transferrin is reported to have a biological half life of about nine days.⁶ A low content of sialic acid on the transferrin molecule has been proposed as a signal to the hepatic cell that the molecule should be eliminated from the bloodstream.^{7,8} This is analogous with findings for other proteins such as orosomucoid.⁹

We have shown that proved alcoholics generally have much increased Tf_{ratio} values with a group mean value more than twice as high as the occupationally exposed workers of this study.³ Alcoholics when in

hospital for more than a week usually showed decreasing Tf_{ratio} s. This has been found to be due mostly to increasing values of Tf_{10} rather than to decreasing $Tf_{5.7}$ (S Petré, O Vesterberg, unpublished observation). For the solvent exposed subjects of this study, Tf_{10} was, on average, 10% higher than for the alcoholics and the values did not change significantly after their holiday. The $Tf_{5.7}$ was lower in the alcoholics and did not change either. A small proportion of the alcoholics who were followed up for 21 days, however, did not obtain Tf_{ratio} values within the normal range (below 3.0) during the study.³ The present study indicates that long term exposure to organic solvents may have an effect on the liver similar to that of alcohol abuse. The effect (higher Tf_{ratio} s) is less pronounced, however, than after alcohol abuse. The exposure to organic solvents in the plant where the studies were carried out has decreased during the past years due to better education of the workstaff in ways of avoiding excessive exposure and much improved air ventilation. The effects observed on the Tf_{ratio} may be caused by higher exposure in the years preceding this study. Exposure to aromatic solvents—for example, toluene—was higher some years ago but has since decreased.

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