

Immunochemical Measurement of Serum Transferrin

—A Study on Correlation between Immunochemical Determination of Serum Transferrin and Chemical Measurement of Serum Total Iron-Binding Capacity—

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INTRODUCTION

Various methods are available for measuring total iron-binding capacity in serum (TIBC) which is an indirect measure of serum transferrin content. All of these methods are cumbersome and have methodological disadvantages such as a high sample amount, possibility for iron contamination of the laboratory ware, unspecificity because of other iron-binding proteins in serum and problems to optimize the method (Koepke, 1965; Williams and Conrad, 1972; Von der Heul et al., 1972; Frazer, 1973; Haeckel et al., 1973; Schmidt et al., 1975; Tsung et al., 1975; Graham and Bates, 1976; Rajamaeki et al., 1979; Seiffert, 1981).

Immunochemical methods have made a direct immunological measurement of serum transferrin possible (Goodman et al., 1958; Wilding and Rollason, 1972; Haeckel et al., 1973; Schmidt et al., 1975; Tsung et al., 1975; Kreutzer, 1976).

The purpose of the present work was to study the correlation between serum transferrin and TIBC values in healthy and anemic persons.

Further to determine reference values of serum transferrin for adults of both sexes and for 2 groups of children at different age.

MATERIALS AND METHODS

The reference material for adults consisted of 70 men and 48 women, all of them were healthy voluntary blood donors; regular blood donors were excluded in the material. The reference material for children consisted of 46 healthy children divided into 2 groups of 4 to 10 and 11 to 15 years, numbers 22, and 24 children, respectively. The age distribution of the adult reference material was 34.5 ± 9.1 years for men and 37.1 ± 13.7 years for women (mean ± 1 SD).

The patient material consisted of 84 adult anemic patients: 45 with iron deficiency anemia (20 men and 25 women) and 39 with anemia of chronic disorders (21 men and 18 women). The diagnoses were confirmed by examining the bone marrow hemosiderin stores (Bainton & Finch 1964). The hemosiderin stores were stained by the Prussian blue reaction (Roth & Finch 1948).

Blood samples from reference material for transferrin and TIBC determinations were collected randomly otherwise the patient samples

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were collected, after overnight fasting, in the morning. Iron free needles and sample container were used with a caution.

Serum transferrin was determined by a single radial immunodiffusion method (Mancini et al 1965). The sample volume was 5 μ l. Antiserum and standards were obtained from Behringwerke AG (Marburg, West Germany). Daily checked control sera were Monitrol, Precinorm- U, Preclip- C, and Seronorm. The analytical day-to-day precision was 7-9%.

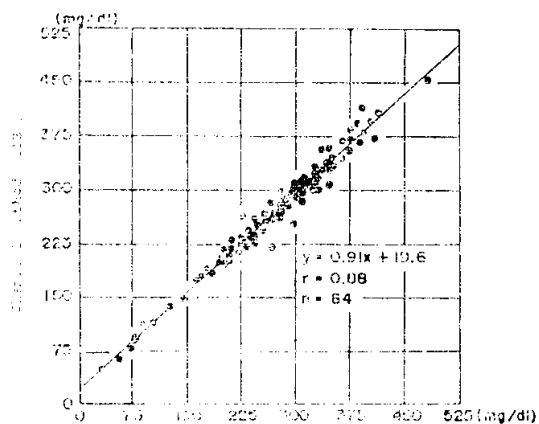
Serum total iron-binding capacity was analyzed with Technicon Autoanalyzer (Giovanniello et al 1967). The sample volume was 1.0 ml. Daily quality control was also done with same commercial sera mentioned above. The analytical day-to-day precision was 6-8%. The adult reference range for serum TIBC measured from the joined reference material was 49-79 μ mol/l for men and 46-71 μ mol/l for women.

For the determination of serum transferrin, We also used the Tina-quant test from Boehringer Mannheim GmbH (Mannheim, West Germany) with LKB 8600 autoanalyzer in addition to check whether it can be performed in routine laboratory (Seiffert et al 1980).

The transferrin values were converted to TIBC equivalents (TIBC equivalent=transferrin value \times 23.0; Tsung et al 1975). We used the common standard statistical methods for method-comparison study: the linear regression equation between serum transferrin and TIBC values.

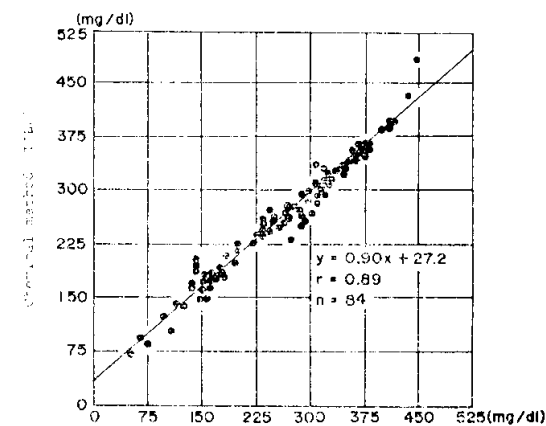
RESULT

The linear regression equation between transferrin and TIBC values were $y=0.91x+19.6$ ($r=0.88$) with a single radial diffusion method and $y=0.90x+27.2$ ($r=0.89$) with a Tina-quant test when the values of TIBC determination(y) were plotted against TIBC equivalents (x). (Figure 1. and 2.).



Single Radial Diffusion (TIBC equivalent)

Fig. 1. Correlation of transferrin (TIBC equivalent) and TIBC values of 84 patients with anemia using a single radial diffusion method for the direct determination of serum transferrin.



Immunologic Turbidimetry (TIBC equivalent)

Fig. 2. Correlation of transferrin (TIBC equivalent) and TIBC values of 84 patients with anemia using the immunologic turbidimetry for the direct determination of serum transferrin.

The reference values for serum transferrin are presented in Table 1. for adults and 2 groups of children. At the age group 11 to 15 years the values are close to adult values. No sex differences could be detected in any of the children groups not so as the adult group.

DISCUSSION

Since the first report on direct immunoche-

mical determination of serum transferrin by Goodman et al (1958), several works have been published discussing this issue (Wilding and Rollason, 1972; Haeckel et al., 1973; Schmidt et al., 1975; Tsung et al., 1975; Kreutzer, 1976). Several workers concluded that immunochemical methods for serum transferrin determination are superior to TIBC determination due to methodological advantages (Von der Heul et al., 1972; Haeckel et al., 1973). On the other hand, Tsung et al. (1975) concluded that transferrin determination offers no advantages over TIBC determination in diagnostic purpose.

A comparison of reference values by several workers with the our results is shown in Table 2. Our reference values for adults are based on a careful selection of healthy voluntary blood donors (no regular blood donors were included in the material), but sample collection was made randomly to compare the data with the samples of overnight fasting. However it agree quite well with the values reported by Tsung et al. (1975) and Rajamaeki et al. (1979).

Our reference values for children at the age group 4 to 10 years are higher than adult values (2.4-3.7 g/l) and for children group at the age of 11 to 15 years close to adult values (2.3-3.3 g/l).

Rajamaeki et al (1979) proposed that the

Table 1. Reference values of serum transferrin for adults and children using Single Radial Diffusion method

| | n | Mean (mg/dl) | Mean±2SD (mg/dl) | Range (mg/dl) |
|----------------|----|--------------|------------------|---------------|
| Men | 70 | 269 | 212-340 | 200-350 |
| Women | 48 | 260 | 201-313 | 200-330 |
| Children | | | | |
| 4 to 10 years | 22 | 302 | 241-362 | 230-380 |
| 11 to 15 years | 24 | 279 | 231-330 | 220-330 |

direct immunochemical determination of transferrin is beneficial to distinguish patients with iron deficiency anemia and with anemia of chronic disorders from normals somewhat better than TIBC determination.

The correlation between the values of transferrin and TIBC determination has been stated to be good in several reports (Tsung et al., 1975; Kreutzer, 1976, Rajamaeki et al., 1979). Our results agree well with these opinion. It appears that a practical advantages of transferrin determination is the smaller sample volume demand especially considering the anemia diagnosis of pediatric patients. There were no significant differences between single radial diffusion method and immunologic turbidmetric test on the point of correlation with TIBC. It appears that a single diffusion method is better for small sized laboratory hence the turbidmetric test is adequate for busy routine laboratory.

Table 2. Comparison of adult reference values for serum transferrin

| | Reference material | Values (mg/dl) |
|-------------------------|--|----------------|
| Wilding & Rollason 1972 | 194 men, health center patients | 161-353 |
| | 72 women, health center patients | 143-359 |
| Tsung et al 1975 | No information | 187-312 |
| Kreutzer 1976 | 51 men and 30 women, 'apparently healthy' | 230-380 |
| Rajamaeki 1979 | 167 men, healthy voluntary blood donors | 210-340 |
| | 167 women, healthy voluntary blood donors | 200-310 |
| The present study | 70 men, healthy voluntary blood donors, randomly sampled | 212-340 |
| | 48 women, healthy voluntary blood donors, randomly sampled | 201-313 |

SUMMARY

Serum transferrin was determined in the anemic subjects using the immunochemical methods for the study on the correlation between it and total iron binding capacity measured chemically. Further, the reference values of serum transferrin for adults of both sexes and for two groups of children at different ages were also studied using the single radial diffusion method.

The linear regression equation between transferrin and TIBC values were $Y=0.91\bar{u}+19.6$ with a single radial diffusion method and $Y=0.09x+27.2$ with a immologic turbidimetry and show excellent correlation with the r value of 0.88 and 0.89, respectively.

The reference values of serum transferrin for adults were 200 to 350mg/dl in male and 200 to 330mg/dl in female healthy subjects. In case of the children, the age group of 4 to 10 years have the reference range of 230 to 380mg/dl and the age group of 11 to 15 years have the its range of 220 to 330mg/dl.

No sex differences could be detected in any of the children groups not so as the adult group of which male subjects have a somewhat higher than that of female subjects in their reference ranges.

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—國文抄錄—

免疫化學測定法을 이용한 血清 Transferrin 測定에 관한 研究

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本論文은 Single Radial Diffusion과 Immunologic Turbidimetry를 利用하여 血清 Transferrin을 직접 測定하는 새로운 免疫化學測定法에 관한 研究이다. 貧血患者群에서 化學的測定法으로 血清 Transferrin을 간접적으로 나타내는 總鐵結合能(TIBC)值를 測定한 후 면역化學測定法으로 測定한 血清 Transferrin值와 비교하였고 正常人에서 얻은 檢體를 Single Radial Diffusion法으로 測定하여 다음과 같은 結果를 얻었다.

1. Single Radial Diffusion法으로 測定한 血清 Transferrin值와 化學的方法으로 測定한 Total Iron-Binding Capacity사이에 좋은 相關關係를 얻을 수 있었다($y=0.9x+19.6$, $r=0.88$, $n=84$).

2. Immunologic Turbidimetry法으로 測定한 血清 Transferrin值와 化學的方法으로 測定한 Total Iron-Binding Capacity사이에도 역시 좋은 相關關係를 얻을 수 있었다($y=0.90x+27.2$, $r=0.89$, $n=84$).

3. 면역化學測定法(Single Radial Diffusion法)을 利用한 血清 Transferrin의 참고치(Reference Values)는 다음과 같다.

가. 正常成人男子 : 200—350mg/dl.

나. 正常成人女子 : 200—330mg/dl.

다. 正常小兒

1. 4세—10세群 : 230—380mg/dl.

2. 11세—15 群 : 220—330mg/dl.

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