Transferrin Polymorphism Influences Iron Status in Blacks


Background: Genetic variants of human transferrin (TF) have been described, but little is known about their functional differences. We studied iron status according to TF phenotype in a healthy Zimbabwean population and in subjects at risk of African iron overload.

Methods: The study population consisted of 483 non-drinkers, 31 drinking spouse pairs, and 5 family pedigrees (n = 88) with index cases of iron overload. TF phenotypes were determined using starch gel electrophoresis. To evaluate iron status, serum iron, total iron-binding capacity (TIBC), ferritin, and soluble TF receptors were measured, and the percentage of saturation and the serum iron:TF ratio were calculated. The binding of the TF variants was studied by equilibrium dialysis.

Results: The reference population was characterized by a high TF D allele frequency (0.050) and a complete absence of homozygous TF DD individuals. Similar allele frequencies were observed in subjects at risk of African iron overload. In the reference population, male TF CD heterozygotes had significantly lower (P < 0.01) values for serum iron, TIBC, TF saturation, and serum iron:TF ratio than the TF CC homozygotes; in females, only TIBC was significantly different. Overall red blood cell indices did not differ according to TF phenotype. In the population at risk of African iron overload, only serum iron:TF ratio was consistently significantly lower in TF CD phenotypes (P < 0.05). After equilibrium dialysis, the amount of iron bound by TF was significantly lower (P <0.01) in TF CD individuals.

Conclusions: The present data demonstrate a functional difference between TF phenotypes in blacks.

Transferrin (TF) is an iron-transporting plasma protein. It has a capacity to bind and transport ferric iron from the intestinal sites of absorption and the sites of hemoglobin breakdown to specific iron-requiring cells and the various sites of iron storage (1).

Genetic variations of human serum TF were first discovered in 1957 by Smithies (2). In addition to the common TF type, which was designated TF C, a series of anodal (B) and cathodal (D) variants have been reported in different human populations (1 –6). In Caucasians, the common allele C is found almost exclusively. West Africa and western stream Bantu has the highest frequencies of (almost complimentary) D allele (7). In US blacks, TF CD phenotype frequency is 10% (4).

The prevalence of iron overload in sub-Saharan Africa is the highest in the world, with reports that up to 10% of some rural populations are affected (8). In these populations, African iron overload has been attributed to high iron intake via the consumption of a traditional alcoholic beverage that is brewed in nongalvanized iron utensils (8, 9). However, recent research has elucidated the importance of genetic factors that affect iron status (10 –12).

Little is known about the functional differences between TF phenotypes. In 1961, Turnbull and Giblett (13) were unable to show functional differences between TF CC and TF CD variants in US blacks. However, this finding was based on a very limited data set and relatively imprecise methods. Further attempts to establish a relationship between TF types, serum TF concentration, and iron-binding capacity in Caucasians have been inconclusive (14, 15). In blacks, studies on the clinical consequences of TF CD phenotype are lacking. In this study, we reexamined iron status according to TF phenotype in the
general population and, in particular, in subjects at risk of African iron overload.

**Materials and Methods**

**SELECTION OF STUDY SUBJECTS**

The investigation was approved by the Medical Research Council of Zimbabwe, and written informed consent was obtained from each subject. The study subjects were recruited from both rural and urban populations. The study subjects were adherents of a religious community, Seventh Day Adventist, which teaches abstinence from all alcoholic beverages, including traditional beer. The participants were questioned regarding symptoms and complications of iron overload. Clinical examinations were carried out on each subject to determine his or her current health status. Thus, a final group of 483 healthy individuals consisting of 193 males (ages, 30–87 years) and 290 females (ages, 35–92 years) with no history of having drunk traditional home-brewed beer or other alcoholic beverages was enrolled into the study.

In addition to the reference population, serum samples (n = 150) were obtained from a rural community (same ethnic group as the reference population) at risk of iron overload because of traditional beer consumption. The group consisted of 31 drinking spouse pairs and 5 family pedigrees (n = 88) with index cases of iron overload. Iron overload is common in rural Africa (10) and is related to the consumption of traditional home-brewed beer, rich in bioavailable iron (9). This group consisted of equal numbers of males and females and has been described previously (16).

**ESTIMATION OF TRADITIONAL BEER CONSUMPTION**

In the subgroup at risk of iron overload, an estimate of the amount of traditional beer consumed over each subject’s lifetime was made, as described previously (12). The average quantity (expressed in liters) of traditional beer consumed over a period of 1 week was multiplied by 52 times the number of years in which the individual had drunk traditional beer. This estimate provides only a broad approximation of lifetime traditional beer consumption because consumption was probably not uniform over time and information was obtained by recollection.

**COLLECTION AND ANALYSIS OF BLOOD SAMPLES**

Blood samples were drawn from fasting individuals in the morning to avoid the transient increase in serum iron potentially seen after ingestion of an iron-rich meal and to avoid diurnal variation in serum iron (17). The blood was collected using the Vacutainer system (Terumo Medical Corp.) into tubes containing K$_2$-EDTA as anticoagulant and tubes containing no anticoagulant. The samples were transported chilled, and hematological measurements were determined immediately after arrival in the laboratory. Serum was separated and kept frozen at −80 °C until analysis.

**Table 1. TF phenotypes and D allele frequency in the reference population and in subjects at risk of iron overload.**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Reference population (n = 483)</th>
<th>Iron overload risk group (n = 150)</th>
<th>Total (n = 633)</th>
<th>D allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF CC, n (%)</td>
<td>435 (90)</td>
<td>134 (89.3)</td>
<td>569 (90)</td>
<td>0.050</td>
</tr>
<tr>
<td>TF CD, n (%)</td>
<td>48 (10)</td>
<td>16 (10.7)</td>
<td>64 (10)</td>
<td>0.053</td>
</tr>
<tr>
<td>TF DD, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.050</td>
</tr>
</tbody>
</table>

*The distribution of TF phenotypes was not different between the two study groups (P > 0.05).*

Serum iron concentration was determined using a Ferrozine®-based colorimetric method on a Hitachi 747 analyzer (Hitachi), and serum TF concentration was determined using an immunoturbidimetric method on the same analyzer. The serum iron:TF ratio was obtained by dividing serum iron (µmol/L) by the TF concentration (g/L), as measured by immunoturbidimetric method. Total iron-binding capacity (TIBC) was determined by a method modified from the one recommended by the International Committee for Standardisation in Hematology (18). Serum ferritin was assayed using an radiomunnoassay (Diagnostic Products). Soluble serum TF receptors were assayed immunonephelometrically on a BN II analyzer (Dade Behring) using commercial antibodies supplied by the manufacturer. TF saturation was calculated as the serum iron:TIBC ratio expressed as a percentage.

TF phenotyping was carried out on starch gel electrophoresis according to Smithies (2). TF CC and TF CD variants were confirmed by capillary zone electrophoresis on a P/ACE 5000 electropherograph (Beckman), using an uncoated fused-silica capillary [47 cm × 50 µm (i.d.); Beckman]. The buffer system of the CEOFIX HRE Kit (Analis) was used. The capillary was rinsed for 1.0 min under pressure with the “initiator” solution, followed by a 2.0-min rinse with the “buffer” solution. Serum was loaded electrophoretically by pressure injection for 1 s. Separation was performed at a constant voltage of 28 kV. The eluate was monitored at 214 nm, and peak areas were calculated.

Apotransferrin was prepared after dialysis of serum containing either TF CC or CD against an 0.5 mol/L acetate buffer (pH 5.1) for 20 h. Iron binding by TF was calculated after equilibrium dialysis against 0.1 mol/L phosphate-buffered saline solution (pH 7.3) containing various amounts of ferric chloride (range, 0–3 µmol/L) in the presence of 0.005 mol/L sodium bicarbonate.

Erythrocyte sedimentation rates were determined by the Westergren method, and a Coulter Counter model T890 analyzer (Coulter Electronics) was used to measure the hematological indices hemoglobin, hematocrit, mean cellular volume, mean cellular hemoglobin, and mean cellular hemoglobin concentration.
Statistical analysis

Values were expressed as mean ± SD, or as median and interquartile range, where appropriate. Gaussian distribution was tested using the Kolmogorov–Smirnov test. Statistical comparison of indirect measures of iron status and hematological indices between TF-type groups was done by analyses of variance with adjustment for estimated lifetime traditional beer consumption. Values for estimated traditional beer consumption were natural logarithm-transformed for the analysis of variance procedures.

Results

We determined the TF phenotypes of 633 individuals, consisting of 483 subjects not consuming alcoholic beverage and who were otherwise apparently healthy (reference population) and 150 subjects at risk of iron overload. The TF phenotype frequencies, TF CC, TF CD, and TF DD, and D allele frequency are summarized in Table 1.

In the reference population (n = 483), a TF CC phenotype was found in 435 (90%) cases, and the TF CD variant was found in 48 (10%) cases. This distribution corresponds to a TF C allele frequency of 0.950 and a TF D allele frequency of 0.050. No homozygous TF DD cases were found. Fig. 1 shows a capillary zone electrophrogram of the various TF phenotypes found.

The reference values for serum iron, TF, TF saturation, TIBC, the serum iron:TF ratio, ferritin, and soluble TF receptors according to TF phenotype and gender are summarized in Table 2. In males, subjects with the TF CD phenotype had markedly lower values for serum iron (P = 0.009), TIBC (P < 0.001), percentage of iron saturation of TF (P = 0.04), and serum iron:TF ratio (P = 0.01) compared with TF CC subjects. Serum ferritin, TF, and soluble TF receptor concentrations were comparable for the two TF phenotypes. In females, only TIBC was significantly different between the TF phenotypes.

In vitro, after equilibrium dialysis the relative binding of iron by TF (immunoturbidimetry) was significantly lower in TF CD heterozygotes compared with TF CC homozygotes: 6.5 ± 0.4 μmol/g for TF CD (n = 4) vs 8.8 ±
Table 3. Iron status indices according to TF phenotype and gender in the population at risk of iron overload.a

<table>
<thead>
<tr>
<th></th>
<th>TF CCb</th>
<th>TF CDc</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Serum iron, μmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>22.0 ± 11.0</td>
<td>20.4 ± 13.2</td>
<td>NS</td>
</tr>
<tr>
<td>Females</td>
<td>20.0 ± 6.6</td>
<td>8.9 ± 7.7</td>
<td>0.006</td>
</tr>
<tr>
<td>Overall</td>
<td>21.1 ± 8.9</td>
<td>14.1 ± 10.7</td>
<td>0.01</td>
</tr>
<tr>
<td>TIBC, μmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>48.2 ± 8.7</td>
<td>47.1 ± 11.0</td>
<td>NS</td>
</tr>
<tr>
<td>Females</td>
<td>52.1 ± 10.3</td>
<td>53.4 ± 7.0</td>
<td>NS</td>
</tr>
<tr>
<td>Overall</td>
<td>50.0 ± 9.8</td>
<td>50.4 ± 8.8</td>
<td>NS</td>
</tr>
<tr>
<td>TF saturation, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>48.6 ± 25.0</td>
<td>47.3 ± 27.4</td>
<td>NS</td>
</tr>
<tr>
<td>Females</td>
<td>39.9 ± 17.7</td>
<td>19.3 ± 13.7</td>
<td>0.003</td>
</tr>
<tr>
<td>Overall</td>
<td>44.4 ± 22.4</td>
<td>31.7 ± 22.0</td>
<td>0.039</td>
</tr>
<tr>
<td>Serum TF, g/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>2.32 ± 0.43</td>
<td>2.70 ± 0.61</td>
<td>NS</td>
</tr>
<tr>
<td>Females</td>
<td>2.55 ± 0.50</td>
<td>2.89 ± 0.38</td>
<td>NS</td>
</tr>
<tr>
<td>Overall</td>
<td>2.44 ± 0.48</td>
<td>2.80 ± 0.49</td>
<td>NS</td>
</tr>
<tr>
<td>Serum iron:TF ratio, μmol/g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>10.1 ± 5.6</td>
<td>7.0 ± 5.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Females</td>
<td>7.7 ± 3.6</td>
<td>4.3 ± 2.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Overall</td>
<td>8.9 ± 4.6</td>
<td>5.7 ± 4.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum ferritin, μg/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>190 (56–666)</td>
<td>36 (34–1637)</td>
<td>NS</td>
</tr>
<tr>
<td>Females</td>
<td>62 (23–185)</td>
<td>88 (62–141)</td>
<td>NS</td>
</tr>
<tr>
<td>Overall</td>
<td>103 (36–449)</td>
<td>86 (36–145)</td>
<td>NS</td>
</tr>
</tbody>
</table>

a The sample consists of 66 males and 68 females carrying a TF CC phenotype and 7 males and 9 females carrying a TF CD phenotype.

b Values are expressed as the mean ± SD or as the median and interquartile range (for ferritin).

c NS, nonsignificant.

d Wilcoxon test.

1.0 μmol/g for TF CC (n = 4) for a free iron concentration of 1.0 μmol/L (P <0.01).

When we compared areas under the curve for TF on capillary zone electrophoresis (Fig. 1), values for the TF CC phenotypes on average were only 58% of the mean value for the heterozygous TF CD phenotypes (P <0.005).

The hematological indices (erythrocyte sedimentation rate, hemoglobin, hematocrit, mean cellular volume, mean cellular hemoglobin, and mean cellular hemoglobin concentration) of the reference population did not differ (P >0.05) according to TF phenotype.

In the population at risk of iron overload, TF CC was found in 134 cases (89.3%) and TF CD in 16 cases (10.7%). Also in this group, no homozygous TF DD phenotype was found. The obtained allele frequencies (0.947 for TF C and 0.053 for TF D) were comparable to the reference population. The median traditional beer consumption was 3725 L (interquartile range, 0–16 630 L). Table 3 summarizes the major iron status markers according to TF phenotype in this group. In males, only the serum iron:TF ratio was significantly lower in the TF CD subjects (P <0.05), whereas in female TF CD individuals, serum iron, TF saturation, and serum iron:TF ratio were significantly lower (P <0.05). Similar to the reference population, serum TF, soluble TF receptors, and ferritin concentrations were comparable for both TF phenotype groups. Interestingly, in the upper tertile of TF saturation (>42%; n = 50), only two subjects (4%) were TF CD variants. The ratio of TIBC to TF was significantly different (P <0.05): 20.4 ± 1.7 μmol/g for TF CC vs 18.1 ± 1.9 μmol/g for TF CD.

Discussion

The observed TF phenotype distribution in this study corresponds to the distribution reported in other African studies (6, 7). Remarkably, no homozygous TF D phenotypes were observed in the studied adult population. Similar findings have been reported in US blacks (4) and in two other African populations in Mozambique (15) and Rwanda (Wieme R, Department of Informatics, University of Ghent, Ghent, Belgium, personal communication). There has been only a single report of homozygous TF D in an Australian aborigine family (3). This finding could indicate that homozygous TF D can be considered a major disadvantage.

In the absence of iron overload, the male subjects heterozygous for TF C and TF D had significantly lower values for serum iron, TIBC, TF saturation, and the serum iron:TF ratio than those carrying the common homozygous TF C phenotype. In females, no significant differences between TF phenotypes were observed in the reference population. The additional variation of physiological iron losses in females might account for the smaller differences observed between the TF phenotypes.

However, in the population at risk of iron overload, although there was tendency for lower values for serum iron and TF saturation in the male TF CD subgroup, only the serum iron:TF ratio was significantly different. A probable reason for this finding could be the result of variations in the amounts of traditional beer consumed by individuals. Furthermore, the sample size (seven males and nine females presenting with a TF CD phenotype) may be insufficient to allow us to detect significant differences. In females, unexpected significant differences were observed for serum iron and TF saturation. One interpretation of this observation in females could be that it is attributable to chance because of the low sample size (nine subjects with TF CD phenotype).

In the vitro binding of iron by TF from CD individuals was shown to be significantly lower than binding by TF from CC individuals. This difference may account for the observed in vivo differences in serum iron measures.

The comparison between capillary zone electrophoresis and immunoturbidimetry showed unexpectedly lower TF concentrations in TF CD subjects when measured by immunoturbidimetry. Because the absorbance at 214 nm shows a close correlation with protein concentration (19), the serum TF concentration may be underestimated by the immunoturbidimetric method in TF CD subjects. Therefore, TIBC is to be preferred over serum TF in black
individuals with a TF CD phenotype for the evaluation of iron status.

Despite the observed differences in iron status indicators, the peripheral blood profile did not differ according to TF phenotype. It may be speculated that in the adult population studied, the amount of iron available for hematopoiesis is sufficient in both major TF phenotypes.

Previous attempts to link TF polymorphism with biological or clinical consequences have been restricted to non-black populations. Because of the low frequency of TF B and TF D mutants in Caucasians (1), no significant functional or clinical differences have been found in that population. Bantu-speaking subjects of southern Africa carrying the TF D allele are characterized by lower indices of serum iron status. The differences in TF saturation are not associated with hematological changes.

In southern African blacks, the TF polymorphism is a result of an important genetic pressure (7). The lack of homozygous TF D individuals in this population and the differences in TF saturation among TF phenotypes support evidence for the biological and clinical importance of the polymorphism. TF polymorphism might play a role in various disturbances of iron metabolism. In African iron overload, heterozygous TF CD individuals might be partly protected from increased iron accumulation.

In conclusion, our findings suggest that there is a need to investigate further the effects of TF polymorphism on various aspects of iron metabolism.

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References