Exploring the iron metabolism in multidrug resistant tuberculosis (MDR-TB) patients in treatment

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ABSTRACT

The iron metabolism plays a key role in the progression of active Tuberculosis. Several studies have shown a link between iron metabolism disorders and active tuberculosis. The aim of this study was to explore the iron metabolism of 100 patients with multidrug-resistant tuberculosis (MDR-TB) treated with second generation anti-tuberculosis drugs. The levels of iron in serum were assessed using atomic absorption spectrometry (AAS) while ferritin and transferrin were determined by imminoturbidimetry methods. The total iron binding capacity and transferrin saturation coefficient were determined from the values of transferrin and iron in the serum. The data showed a significant decrease in values of serum iron and total iron binding capacity (TIBC), in contrast to ferritin and the coefficient of transferrin saturation (CTS) which were in normal range before or after 6 months of treatment. These results are characteristic of inflammatory anemia. The persistence of this anemia despite treatment requires effective management of the inflammatory process in parallel with the use of second line anti-TB drugs.

Keywords: Iron Metabolism, serum iron, transferrin, ferritin, MDR-TB.

INTRODUCTION:

Iron is the first and the most important trace element in cellular metabolism. It occurs at many metabolic mechanisms including the modulation of the immune system and more particularly in the production of hemoglobin by the process of erythropoiesis. Iron metabolism control occurs at the level of the body to maintain the stock of iron and its distribution to adequate levels, but also at the cellular level, thereby ensuring optimal biological functions (Loreal et al., 2012). Iron deficiency is the cause of various metabolic disorders whose final stage is anemia. While an iron overload could increase 3 to 5 times the risk of developing active tuberculosis (Edem et al., 2015).

Several studies have shown an association disorder of iron metabolism with patients with active tuberculosis (Mc Dermid et al., 2013; Gribel et al., 2014). Other studies reported that the anemia observed in
tuberculosis is due to chronic inflammation caused by the bacillus *Mycobacterium tuberculosis* (Chen et al., 2010). Moreover, according Aouam et al. (2007), some anti-TB drugs would be responsible for hemolytic anemia. Thus, to improve the program of the fight against TB in all its forms in Côte d’Ivoire, the present study aimed at assessing the impact of *Mycobacterium tuberculosis* and the second generation anti-TB on the iron metabolism of MDR -TB patients in current treatment.

**MATERIALS AND METHODS**

**Sampling and sample pretreatment**

The biological material consists of serum of MDR-TB in current treatment. In total, 100 MDR-TB patients were selected for this study. The sampling was carried out in different stages: M₀ stage for the initial assessment before starting treatment and M₁ and M₆ stages for monitoring assessment after 3 and 6 months of treatment. At the end, 300 MDR-TB samples and 100 samples of non-tuberculous voluntary witnesses with as many women as men were selected. It should be noted that all the MDR-TB patients and TB voluntary witnesses are aged between 18 and 55 years.

Samples were taken after an overnight fast in dry tubes (Vacutainer®) and then centrifuged at 3000 rounds/min for 5 min. The serum collected in tubes Ependorf® was subsequently stored at -20 °C.

**Methods for determination of markers of iron metabolism**

This study consists of determining the concentrations of serum iron, serum ferritin, the Total Iron Binding Capacity (TIBC) and the Coefficient of Transferrin Saturation (CTS). The dosage of serum iron was performed using atomic absorption spectrometer (AAS) flame air / acetylene brand Varian AA 20 ® Pattern, France, after deproteinization of the serum by Chloridric acid CARLO ERBA Reagents 2 M (Yaméogo, 2009). The wavelength characteristic of the serum iron was 249 nm. The threshold of detection was 0.001 mg/L. Ferritin and transferrin were determined by immunoturbidimetry methods with Cobas C311 Roche Diagnostic France, a method firstly described by Heidelberger (Heidelberger et al., 1935; Dubois et al., 1988). The dosage principle was based on the fact that human ferritin agglutinates the latex particles coated with anti-ferritin monoclonal antibodies while human transferrin forms a precipitate in the presence of a specific antiserum. Both of the complexes (ferritin with anti-ferritin monoclonal antibodies and the precipitates) obtained were measured by turbidimetry at 340 nm and 552 nm for transferrin and ferritin, respectively. The total iron binding capacity (TIBC) and the coefficient of transferrin saturation (CTS) are calculated from both the values of serum transferrin and serum iron by the following formulas (Wanger, 2000):

\[
\text{TIBC (µmol/L)} = \text{transferrin (g/L)} \times 25.
\]

\[
\text{CTS (µg/L)} = \left(\frac{\text{Iron}}{\text{TIBC}}\right) \times 100.
\]

**Statistical analysis**

The data of iron metabolism were expressed as average values accompanied by the standard error of the mean (mean ± SEM). Statistical analysis of results was performed using analysis of variance (ANOVA) followed by Tukey multiple comparison test. The difference was significant when p-value < 0.05.

**RESULTS**

Serum iron and total iron binding capacity (TIBC) in MDR-TB was very low at the initial stage (M₀) and after 3 and 6 months treatment (M₃ and M₆) for both men and women compared with controls and normal values, P < 0.05. However, a slight increase in values of these parameters was found during the treatment, P < 0.05. The concentration of iron was in mean: 10.7 ± 0.5 µmol/L; 11.7 ± 0.5 µmol/L and 12.5 ± 0.6 µmol/L at M₀, M₃ and M₆ stages respectively against 17.7 ± 1.3 µmol/L in men witnesses and in women: 8.26 ± 0.48 µmol/L; 9.28 ± 0.5 µmol/L and 10.43 ±
0.9 μmol/L at M₀, M₃ and M₆ stages respectively against 17.50 ± 1.1 μmol/L in women witnesses. The values of total iron binding capacity in mean was 41.4 ± 1.1 μmol/L; 47.1 ± 0.8 μmol/L and 47.5 ± 1.2 μmol/L and in women 28.95 ± 1.3 μmol/L; 36.56 ± 1.4 μmol/L and 42.62 ± 2.0 μmol/L at M₀, M₃ and M₆ stages respectively against 63.8 ± 2.1 in men and women witnesses respectively. Regarding ferritin and the coefficient of transferrin saturation (CTS), the values remained in the normal range either in men or in women but with a minimal increase with treatment. All these values, classified by sex, are given in Tables 1 and 2 and Figures 1, 2, 3 and 4 reported the comparative analyses by sex.

Table 1: Values of iron metabolism parameters in men.

<table>
<thead>
<tr>
<th></th>
<th>Men MDR-TB</th>
<th>Men witnesses</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M₀</td>
<td>M₃</td>
<td>M₆</td>
</tr>
<tr>
<td>Iron (μmol/L)</td>
<td>10.7 ± 0.5*</td>
<td>11.7 ± 0.5*</td>
<td>12.5 ± 0.6*</td>
</tr>
<tr>
<td>FERR (μg/L)</td>
<td>201.3 ± 21.5</td>
<td>148.1 ± 11.9</td>
<td>152.7 ± 9.9</td>
</tr>
<tr>
<td>TIBC (μmol/L)</td>
<td>41.4 ± 1.1*º</td>
<td>47.1 ± 0.8*</td>
<td>47.5 ± 1.2*</td>
</tr>
<tr>
<td>CTS (%)</td>
<td>23.6 ± 1.2</td>
<td>24.3 ± 1.0</td>
<td>24.8 ± 1.2</td>
</tr>
</tbody>
</table>

The values of iron metabolism parameters in mean. MDR-TB = Multidrug resistant tuberculosis. TIBC = total iron binding capacity. CTS = Coefficients of transferrin saturation. M₀ = Initial assessment before starting treatment. M₃ or M₆ = tracking sheet after 3 or 6 months of treatment. * Significant difference between MDR-TB and the Witnesses and normal values. P < 0.05. º Significant difference between M₀ (before treatment) and M₃, M₆ (3 or 6 months after treatment). P < 0.05.

Table 2: Values of iron metabolism parameters in women.

<table>
<thead>
<tr>
<th></th>
<th>Women MDR-TB</th>
<th>Women witnesses</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M₀</td>
<td>M₃</td>
<td>M₆</td>
</tr>
<tr>
<td>Iron (μmol/L)</td>
<td>8.26 ± 0.48*</td>
<td>9.28 ± 0.5*</td>
<td>10.43 ± 0.9*</td>
</tr>
<tr>
<td>FERR (μg/L)</td>
<td>168.00 ± 12.0*</td>
<td>164.3 ± 14.6*</td>
<td>115.6 ± 13.0</td>
</tr>
<tr>
<td>TIBC (μmol/L)</td>
<td>28.95 ± 1.3**</td>
<td>36.56 ± 1.4*</td>
<td>42.62 ± 2.0*</td>
</tr>
<tr>
<td>CTS (%)</td>
<td>29.40 ± 1.7</td>
<td>25.52 ± 1.4</td>
<td>24.13 ± 2.2</td>
</tr>
</tbody>
</table>

The values of iron metabolism parameters in women. MDR-TB = Multidrug resistant tuberculosis. TIBC = total iron binding capacity. CTS = Coefficients of transferrin saturation. M₀ = Initial assessment before starting treatment. M₃ or M₆ = tracking sheet after 3 or 6 months of treatment. * Significant difference between MDR-TB and the Witnesses and normal values. P < 0.05. ** Significant difference between M₀ (before treatment) and M₃, M₆ (3 or 6 months after treatment). P < 0.05.
**Figure 1:** Concentrations of Iron by sex. The iron concentrations by sex. MDR-TB = Multidrug resistant tuberculosis. M₀ = Initial assessment before starting treatment. M₃ or M₆ = tracking sheet after 3 or 6 months of treatment.

**Figure 2:** Concentrations of Ferritin by sex. The ferritin concentrations by sex. MDR-TB = Multidrug resistant tuberculosis. M₀ = Initial assessment before starting treatment. M₃ or M₆ = tracking sheet after 3 or 6 months of treatment.
Figure 3: Total Iron Binding Capacity by sex. The values of total iron binding capacity by sex. MDR-TB = Multidrug resistant tuberculosis. $M_0$ = Initial assessment before starting treatment. $M_3$ or $M_6$ = tracking sheet after 3 or 6 months of treatment.

Figure 4: Coefficients of Transferrin Saturation by sex. The values of coefficients of transferrin saturation by sex. MDR-TB = Multidrug resistant tuberculosis. $M_0$ = Initial assessment before starting treatment. $M_3$ or $M_6$ = tracking sheet after 3 or 6 months of treatment.
DISCUSSION

Iron status plays an important role in the progression of active TB (Kassu et al., 2006). Exploration of iron metabolism comprises the determination of serum iron that represents the pool of exchanges, ferritin reflects the reserve pool and finally transferrin and total capacity of iron binding to transferrin that represents the pool of functional iron (Mario, 2012). The present study showed that serum iron and total iron binding capacity (TIBC) were significantly lower in highly multiresistant tuberculosis (MDR-TB) compared with non-tuberculous witnesses and normal values while the serum ferritin rate and coefficient of transferrin saturation (CTS) remained in the normal range. These results are characteristic of inflammatory anemia or anemia of chronic diseases as previously reported (Celi et al., 2011; Gavazzi, 2014). This type of anemia is not due to an iron deficiency but rather a functional deficiency of iron. Our results are in accordance with those of Lovey et al. (2010) that have shown that serum ferritin, greater than 100 mg/L, excludes the hypothesis of iron deficiency and that the coefficient of transferrin saturation (CTS) only decreases when iron stores are completely exhausted. However, low serum iron concentrations and the decrease in iron total binding capacity are explained by a strong iron retention in macrophages and the decrease of the supply of iron to erythropoiesis during chronic inflammation induced by mycobacterial infection (Beaumont and Karim, 2013). The mechanisms leading to the introduction of this type of anemia are set through the production of various cytokines including interferon-γ, TNF-α and interleukins 1 and 10 that cause the iron sequestration by macrophages and repress the erythropoietin synthesis. This process is amplified by excessive synthesis of hepcidin. A pro-inflammatory protein produced mainly by hepatocytes and secreted into the blood stream interacts with ferroportin which is the exporter of the iron present in enterocytes and macrophages causing the degradation there of. This leads to a decrease in intestinal iron absorption and to a retention of iron in macrophages and hepatocytes (Celi et al., 2011). This set of mechanisms helps to reduce the concentration of serum iron and iron total binding capacity of transferrin (Marioa, 2012). However, there was a slight increase in these values during processing. These results are contrary to the work of Edem et al. (2015) which showed a progressive decrease in iron concentrations in TB patients currently treated. Because tuberculosis molecules of second intensity anti-TB drugs have no hemolytic activity in MDR-TB unlike those of the first generation such as Rifampicin (Aouam et al., 2007). There is no significant difference between men and women in MDR-TB. This indicates that the disorder in iron metabolism is not linked with sex.

Conclusion

It appears from this study that MDR-TB patients have an inflammatory type of anemia characterized by lower concentrations of serum iron and total iron binding capacity. The persistence of this anemia despite the treatment provided to these MDR-TB requires effective management of the inflammatory process in parallel with the use of second generation anti-TB. However, other studies such as the dosage of hepsidine and soluble transferrin receptor (STfR) are seen in order to confirm the inflammatory origin of the anemia.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS

This work was carried out in collaboration between all the authors. APB carried out the study, the statistical analysis and prepared the manuscript; GAB carried out the technical analysis and prepared various parts of the manuscript; SM corrected the English version of the manuscript; AJD co-directed the study; All the authors read and approved the final manuscript.
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