Title: Haptoglobin phenotype and soluble transferrin receptor among tuberculosis

Abstract

Introduction: The antioxidant property of haptoglobin (Hp), variable according to the phenotypes, is associated with the pathogenesis of various diseases. Phenotype of haptoglobin is associated with iron metabolism, which is essential for the growth of Mycobacterium tuberculosis. A potential link between Hp phenotype and blood soluble transferrin receptor (sTfR) has not yet been established. This study aimed to determine the association between Hp phenotype and blood sTfR during tuberculosis (TB).

Methods: With a case-control study involving 70 TB patients and 70 healthy subjects were performed phenotyping of Hp by electrophoresis in polyacrylamide gel and determination of blood sTfR by immunoturbidimetry. Results: The 3 major phenotypes of Hp were found: Hp1-1, Hp2-1, Hp2-2. Hp2-2 phenotype was less common among TB patients (17.4% vs 82.6%, \( p < 0.001 \)). The average rate of TB sTfR was lower than that of healthy subjects (27.5 % vs 5.7 %, \( p = 0.014 \)). In this group, blood sTfR of \( Hp^{2} \) subjects (Hp2-1 and Hp2-2) was lower than those of \( Hp^{1} \) subjects (\( p = 0.011 \)). However, in healthy subjects, it appeared no association between Hp phenotype and blood sTfR. Conclusion: the percentage of Hp2-2 was lower in patients with tuberculosis. Furthermore, Hp1-1 phenotype and \( Hp^{1} \) genotype were the most frequent among tuberculosis. In spite of \( M. \) tuberculosis infection, iron-dependent, no association was found between Hp and iron deficiency.

Keywords: Phenotype – Haptoglobin – Soluble transferrin receptor – Mycobacterium tuberculosis – Tuberculosis
Introduction

Tuberculosis is an infection which prognosis is associated with specific factors of the host [1; 2]. Haptoglobin (Hp) is a glycoprotein with 3 major phenotypes: Hp1-1, Hp2-1 and Hp2-2 [3]. Previous studies showed that those phenotypes could be prognosis factors in different pathologies [4; 5]. Hp2-2 phenotype less antioxidant, could be associated with an increased morbidity and mortality among TB [6].

The association between Hp phenotype and the body iron pool has been established. In some infections such as HIV/AIDS, Hp2-2 phenotype would be associated with a preserved iron pool but with a shorter survival [7].

Iron is required for the growth of bacteria including Mycobacterium tuberculosis (MTb) and consequently impact the prognosis of TB [8]. Besides, the soluble transferrin receptor (sTfR) a good marker of iron pool [9]. Therefore, it could exist an association between Hp phenotype and pool of iron during tuberculosis, an iron-dependent infection.

Our study aimed to determine the association that might exist between Hp phenotype and the iron status marker sTfR during TB.

Material and methods

Study subjects

A case-control study was conducted on 140 male and female adult subjects (70 TB patients group and 70 healthy subjects group) aged within 18 to 60 years. They were respectively followed at the tuberculosis center of Treichville, Abidjan (CATT) and the National Blood Transfusion Center (CNTS), both in Abidjan (Côte d’Ivoire). CNTS subjects have done a medical examination to rule out any possible pathology. The study was conducted after obtaining written informed consent from all subjects.

Analytical methods

Blood samples were collected on heparin tubes and transported to the laboratory (CeDReS). Phenotyping of Hp was performed by electrophoresis in 5% polyacrylamide gel according to Raymond [10]. Pool of iron was assessed through blood sTfR and measured by immunoturbidimetry on Roche COBAS C311 analyzer [11]. The allelic genotype, \(Hp^1\) or \(Hp^2\) was deducted from the phenotypes: genotype \(Hp^1\) from phenotype Hp1-1 and genotype \(Hp^2\) from phenotype Hp1-2 or Hp2-2 [12].
Statistical methods

Distributions were described with effectives, percentages, means and standard deviations. The comparison of nominal variables (Hp phenotypes, alleles, health status) was made with Pearson’s chi-square test (or Fisher's test according to the distribution of the variables). The comparison of quantitative variables (sTfR) for non-Gaussian distributions was made with Mann-Whitney’s non-parametric test or Kruskal-Wallis’s test according to the number of distribution (SPSS v18). Statistical significance was accepted for $p$-value < .05.

Results

Studied groups (healthy subjects and TB patients) were homogeneous in terms of age (Table I).

Table I: Characteristics of study populations

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects</th>
<th>TB patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effective</strong></td>
<td>69</td>
<td>70</td>
</tr>
<tr>
<td><strong>Sex male</strong> (% within group)</td>
<td>54 (78%)</td>
<td>51 (73%)</td>
</tr>
<tr>
<td><strong>Age in years</strong> (mean ± standard deviations)</td>
<td>35 ± 1,1</td>
<td>35 ±1,5</td>
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</table>

TB: tuberculosis

1. Hp phenotype and genotype

The three major phenotypes of Hp (Hp1-1, Hp2-1 and Hp2-2) were found in our populations. Hp0-0 phenotype was not found. Hp2-2 phenotype was less frequent among TB patients than in healthy group (17.4% vs 82.6%, $p < .001$). In the TB group, the probability for Hp1-1 compared to Hp2-2 was 3.52 fold higher (Fig 1). \(Hp^l\) genotype was more frequent among TB (relative risk = 1.47) (Tab II).
% intra-group; Chi\(^2\) test, \(p = .014\)

Odds Ratio TB for Hp1-1/Hp2-2 = 3.52

Figure 1: Distribution of subjects according their health status and their Hp phenotype
Table II: Haptoglobin genotype according to health status

<table>
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<tr>
<td><strong>Effective</strong></td>
<td>69</td>
<td>70</td>
</tr>
<tr>
<td>(Hp^1) (% within group)</td>
<td>24 (38%)</td>
<td>38 (54%)</td>
</tr>
<tr>
<td>(Hp^2) (% within group)</td>
<td>45 (65.5%)</td>
<td>32 (45.7%)</td>
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*TB: tuberculosis; Relative risk Health status = TB for \(Hp^1\) is 1.47 IC\(_{95}\%\) = [1.06 – 2.05]*

2. Soluble transferrin receptor

2.1 Soluble transferrin receptor and haptoglobin phenotype

The average rate of sTfR among TB patients was lower than that in healthy subjects (3.69 ± 0.11 vs 4.24 ± 0.15 mg/l; \(p = .004\)). The mean was ranged within the normal values (2.2 - 5 mg/l in men and 1.2 - 4.4 mg/l in women). Healthy subjects had the same sTfR means whatever was the Hp phenotype. However, the mean sTfR in TB patients with Hp1-1 was significantly higher than that of Hp2-1 subjects (\(p = .020\)) (Fig 2).
In healthy group, there was no significant difference in sTfR according to Hp genotype ($p > .05$). However, in TB group, $Hp^1$ allele was associated with a higher mean of sTfR (3.97 ± 1.00) than $Hp^2$ allele ($p = .011$) (Figure 3).
Figure 3: Distribution of blood sTfR according the health status and the Hp genotype

Discussions

Some studies established an association between Hp phenotype and iron status [13, 17]. Other studies stated an association between Hp phenotype and TB onset or its prognosis [6]. But, none study has established the relationship between Hp phenotype and iron status during an iron-dependent mycobacteria infection. Therefore, we determined the nature of these associations which have prognostic and medical care value.

We used immunoturbidimetry method for its analytical performances sufficient for diagnosis and prognosis [10]. Given the absolute association between Hp genotype and Hp phenotype, we chose Hp phenotyping which present the expected analytical performance with a low cost [14].
Distribution of Hp phenotypes within the studied population

The general distribution of Hp phenotypes among healthy subjects was significantly different from the one among TB group. Recent studies in tuberculosis showed that the Hp2-2 phenotype that is less antioxidant, appears as a less protective marker. It is associated with an increased morbidity and mortality during TB [6]. But, in our study, Hp2-2 phenotype was less frequent in the TB group (17.4% vs 82.6%; \( P < .001 \)) (Figure 1). This could be due to a lower susceptibility of Hp2-2 phenotype to be infected by MTb. Or it could be a survivorship bias, consequence of a higher mortality of Hp2-2 in TB group. Such a bias could be confirmed in a longitudinal study. So in our study, the Hp1-1 phenotype is the one which was most common among TB (odds ratio Hp1-1/Hp2-2 = 3.52). Therefore Hp phenotype is associated with the susceptibility to TB in contrary with the observations of Kasvosve [6]. Moreover, the allele \( H p^1 \) was most associated with TB infection (odds ratio \( H p^1/Hp^2 = 1.47, 95\% CI = [1.06 - 2.05] \)). These results are similar to those of Allison in 1958 by which the susceptibility to TB was associated with Hp genotype within an Ivorian population (70% for \( Hp^1 \)) [15]. But the distributions of phenotypes retrieved in our populations (Fig 1) are different from Allison’ones in 1958: 49% for Hp1-1, 42% for Hp2-1 and 9% for Hp2-2 [15]. These differences would have appeared along time with migration of populations. That promoted genetic mixing.

Soluble transferrin receptor based on health status

The mean of sTfR was statistically different according the group of population. The mean of sTfR in TB group was significantly lower than the one in healthy subjects (\( p = .004 \)). The sTfR varied according the group of population. Nevertheless, sTfR in TB group (3.69 ± 0.11 mg/l) ranged within the values expected for healthy subjects [16]. This means that the TB patients had no iron deficiency. Despite iron is used by MTb for his growth, this use remains below the body needs and does not lead to an iron deficiency.

Soluble transferrin receptor based on Hp phenotype

The mean of sTfR in healthy subjects revealed no significant differences according to Hp phenotypes (Fig 2). The sTfR is not associated with Hp phenotype in healthy subjects. What is contrary with what Delanghe reported [17]. However, our results are similar with those of Kasvosve who found that among the black healthy African, iron status is not affected by Hp
phenotype [18]. Recent studies have shown that the susceptibility of patients to infectious
diseases and the prognosis, including TB, varied according to their Hp phenotype [4-6]. This
was confirmed by our study with a sTfR varying according Hp phenotype in TB group. In our
study, Hp1-1 phenotype ($p=0.020$) and $Hp^1$ allele ($p=0.011$) could be associated with higher
sTfR in TB. This means that in case of TB, Hp1-1 patient could need more iron. Hp2-2
subjects, less common in the group of TB patients seem have a preserved iron pool.

Conclusion

This study retrieved 3 phenotypes within our populations: Hp1-1, Hp2-1 and Hp2-2. Their
distribution among healthy subjects was significantly different from TB group. MTb infection
did not induce iron deficiency whatever is Hp phenotype. Nevertheless, the $Hp^2$ allele
(leading to phenotypes Hp2-1 and Hp2-2) could be associated with a lower sTfR blood
concentration. Phenotype Hp2-2 appears less common among TB patient and the $Hp^1$
genotype seems to present a higher risk to be infected with MTb. These results should be
confirmed in a longitudinal study with a cohort in order to exclude any survivorship bias.

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