Hematological parameters in Pulmonary Tuberculosis patients with and without HIV infection

Kuppamuthu Ramakrishnan*, AlaguVengatesan*, Rajaiyah Shenbagarathai†, Karuppusamy Kavitha and Ponniah Thirumalaikolundusubramanian†

National Institute for Research in Tuberculosis*, Chennai, †Department of Medicine, Madurai Medical College, Department of Biotechnology, Lady Doak College, Madurai †, 37th Day School, Madurai, and ‡ Medicine Department, Chennai Medical College Hospital and Research Centre, Irungalur, Trichy.

ARTICLE INFO

Keywords:
Hematological abnormalities
HIV
Pulmonary tuberculosis
Anemia

ABSTRACT

Hematological parameters are altered during infections. When dual infections such as HIV and pulmonary tuberculosis (PT) occur together, changes are bound to happen. The present study was undertaken in HIV infected patients with and without PT to assess their hematological status and to compare it with that of healthy family members without any infection or illness. The study included 162 participants, of whom 42 were positive for both HIV and TB (group A), 32 HIV negative but TB positive (group B), 45 HIV positive but TB negative (group C), and another healthy 43 were kept as control who were negative for both diseases (group D). Standard methods were adopted to diagnose the diseases. Hematological status was assessed by two pathologists independently and average of the two was taken for analysis. Hemoglobin levels (Hb), total RBC, and PCV were low in all groups without significant differences between the groups. On the other hand, total WBC was significantly lower (P< 0.05) in the HIV infected groups irrespective of the presence of coexisting tuberculosis infection. TWBC and ALC correlated positively with each other in all groups. ESR was significantly elevated (P <0.001) in groups A, B and C as compared to healthy controls (group D). In view of the hematological alterations, practitioners should constantly monitor the hematological status of these individuals and provide appropriate supportive measures.

1. Introduction

Hematological abnormalities were described in patients with mycobacterial infections for almost 100 years and findings may be minimal or profound (1). Human Immunodeficiency Virus (HIV) is one of the strongest known risk factor for the development of tuberculosis (TB) (2). TB may occur at any time after HIV infection, but becomes more common as the immune system weakens (3). Cytopenia is a common complication of infection with human HIV type-1, and during the course of the disease more than 70% of the patients develop anaemia, frequently requiring blood transfusion (4). Neutropenia, lymphopenia and thrombocytopenia were often noticed in HIV infected individuals, indicating that more than one haematopoietic lineage may be impaired (5). These manifestations also reflect the underlying immune status if interpreted cautiously, especially if the patient is being regularly followed up (6). When both infections coexist, the hematological abnormalities may get exaggerated. Hence, the present study was undertaken to assess the status of hematological abnormalities in pulmonary tuberculosis (PT) patients with and with out HIV infection.

Materials and Methods

Study population

Smear positive, newly diagnosed pulmonary TB patients attending the TB clinic at Government Rajaji Hospital, Madurai, India, were recruited to the study after obtaining informed consent. The study was approved by the institutional ethics committee. Briefly, a patient was defined as smear positive if two out of three morning sputum samples were positive for Acid Fast Bacilli (AFB), or if one sputum sample was positive, and chest X-ray and clinical symptoms were suggestive of pulmonary tuberculosis (7). Exclusion criteria were hospitalization or signs of any concomitant chronic or acute infectious disease other than TB/HIV on clinical examination. All patients were free from bleeding manifestations, antiretroviral therapy, endocrine disorders, other organ dysfunction or systemic disorders and chronic inflammatory diseases. These patients were not taking any...
vitamins or hematinics at the time of the study. Healthy members belonging to the patients’ family and living together with them, but free from illness or consuming hematinics were recruited as control. Women included in the present study were neither pregnant nor breast feeding.

**Sample collection and processing**

Fasting blood samples were collected in two EDTA-coated vacutainers from all subjects via venepuncture in the morning. HIV infection was diagnosed by performing two ELISA tests with two different diagnostic kits (Inno test, Belgium and Lab system, Finland) according to WHO’s recommendation for developing countries. A patient was designated as HIV positive if both tests were positive. Hematological tests such as total red blood cells (RBCs) and total white blood cells (TWBCs), were counted using an improved Nuebauer counting chamber, hemoglobin (Hb) levels were determined using cyanmethaemoglobin colorimetric method, erythrocyte sedimentation rate (E.S.R.) using Westergran method, and packed cell volume (PCV) using the standard procedure. Peripheral smears were made on clean glass slides and subjected to Leishman’s stain for differential count (DC). All hematological parameters were analyzed and scored independently by two pathologists and the average of the two readings was taken for analysis. Absolute lymphocyte count (ALC) was calculated based on the total WBC count.

All laboratory procedures were performed following good laboratory practice and using established standard operational procedures (SOPs) (B). All hematological parameters investigated were subjected to internal quality assessment.

**Statistics**

One-sample Kolmogorov-Smirnov test was used to check whether the data were normally distributed. Mean and standard deviation (SD) were used for reporting normally distributed variables. ANOVA was applied for the variables such as TWBC, ALC, Hb, RBC and PCV. The SPSS software package (Windows version 14, SPSS, Chicago, IL) was used for all statistical analyses and a P-value of < 0.01 was considered significant. Kruskal-Wallis test was applied to analyze the significance of ESR status (non-normally variable) and to compare it with different groups.

**Results**

Of the 163 subjects included in the study, 120 were patients and 43 were healthy controls. The median and mean age of the patients and controls were 34 and 36 years respectively. The patient population was classified into three groups: Group A comprising of patients with both HIV and TB (n =43: M=35, F=8), Group B comprising of with HIV negative TB cases (n = 32: M=21, F=11) and Group C comprising of HIV positive TB negative cases (n = 45: M=29, F=16). Healthy controls free from HIV and TB infection were categorized as Group D (n =43: M=22, F=21). Healthy controls were age matched. TWBC, ALC, Hb, RBC, PCV and ESR were determined for all individuals (Table 1). ALC and TWBC were found to correlate positively with each other in all groups. There was no significant difference in other parameters such as Hb, RBC count and PCV. Irrespective of the status of infection i.e., dual or single. ESR was significantly elevated (P < 0.001) in groups A, B and C compared to healthy controls (group D).

**Table 1. Statistical Analysis carried out**

<table>
<thead>
<tr>
<th>Test carried out</th>
<th>HIV (+)/TB (+) (A)</th>
<th>HIV (-)/TB (+) (B)</th>
<th>HIV (+)/TB (-)(C)</th>
<th>HIV (-)/TB (-)(D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>Mean</td>
<td>STD</td>
<td>Mean</td>
<td>STD</td>
</tr>
<tr>
<td>TWBC</td>
<td>6006-6905</td>
<td>6455</td>
<td>7474-8881</td>
<td>8178</td>
</tr>
<tr>
<td>Hb</td>
<td>8.9-9.9</td>
<td>9.4</td>
<td>8-9.7</td>
<td>9.0</td>
</tr>
<tr>
<td>RBC</td>
<td>2.9-3.2</td>
<td>3.1</td>
<td>2-3.2</td>
<td>2.9</td>
</tr>
<tr>
<td>PCV</td>
<td>26-29</td>
<td>28</td>
<td>25-29</td>
<td>27</td>
</tr>
<tr>
<td>ALC</td>
<td>1085-4316</td>
<td>2560</td>
<td>680-5527</td>
<td>2849</td>
</tr>
</tbody>
</table>

**Table 2. Statistical Analysis carried out**

<table>
<thead>
<tr>
<th>Test carried out</th>
<th>HIV (+)/TB (+) (mean) vs. Control (mean)</th>
<th>HIV (-)/TB (+) (mean) vs. Control (mean)</th>
<th>HIV (+)/TB (-) (mean) vs. HIV (-)/TB (-) (mean)</th>
<th>HIV (+)/TB (+) (mean) vs. HIV (+)/TB (-) (mean)</th>
<th>HIV (+)/TB (+) (mean) vs. HIV (+)/TB (-) (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WBC</td>
<td>P &gt; 0.05;</td>
<td>P &lt; 0.05; S.D.</td>
<td>P &gt; 0.05; N.S.D.</td>
<td>P &lt; 0.05; S.D.</td>
<td>P &lt; 0.05; N.S.D.</td>
</tr>
</tbody>
</table>
Discussion

HIV has affected 60 million victims globally, and in India, this figure is around 2.4 million. The clinical spectrum of HIV in India is different from the rest of the world. And there is a great variability within India as well. Hematological abnormalities are the second commonest cause of morbidity and one of the common causes of mortality in HIV patients. Various studies have been carried out on HIV/AIDS in India, studies focusing on the hematological manifestations of the disease are limited, and socio-economic status might have also contributed to these abnormalities, and a synergistic effect of dual infection on the same.

White blood corpus cell count

The total WBC count was significantly higher (p<0.01) in those with isolated TB (Group 'B') than in those with dual infection (Group 'A'), or isolated HIV infection (Group 'C') as well as in healthy controls (Group 'D'). Amilo et al. (18) also reported statistically higher TWBC among PTB patients. Amilo et al. 2001 reported that there is a statistically higher total WBC count among PTB and PTB-HIV coinected patients. In contrast, a study from Nigeria Awodu (19) demonstrated a fall in TWBC among TB patients. As the response of bone marrow to M. tuberculosis infection varies in different individuals, one may have to attribute this to genetic status and ethnic variations of patients, as well as the infectious nature of the pathogens. The drop in TWBC count in patients with isolated HIV infection is due to the suppressive effect of HIV infection on the marrow, affecting production of WBC.

Absolute lymphocyte count

TB may independently suppress lymphocyte number, thereby worsening HIV-related immunosuppression. As treatment appears to reverse this trend, early diagnosis of TB is important. Although serial absolute lymphocyte counts are not very stable, they were found to correlate well with CD4 lymphocyte counts in HIV-seropositive individuals suggesting that absolute lymphocyte count may be used as a crude measure of disease progression during HIV infection. Other investigators from India reported that absolute lymphocyte count (ALC) could serve as an inexpensive laboratory indicator to help physicians predict treatment failure and also as a useful marker for staging disease and predicting progression to AIDS. A decline in ALC may indicate advanced HIV infection, reflect depletion of CD4 T lymphocytes, enhanced immunosuppression and anemia.

Hemoglobin level

Anemia is the most commonly encountered haematologic abnormality in HIV positive patients, which increases as the disease progresses. Several factors play a role in the development of anemia in patients with HIV, and those are chronic infections, associated opportunistic infections, enteropathy and nutritional deficiencies. In this study, the Hb level was low in all groups irrespective of the infectious status (9.4, 9.0, 9.3 and 9.8 g/dl in groups A, B, C and D respectively). Lower levels of Hb have also been noticed in many studies including those from India. The cause of anemia among patients and controls belonging to the low income group is found to be related to inadequate nutrient intake due to low purchasing power, ignorance, illiteracy, influence of elders on diet, superstitious beliefs, and underutilization of nutritional supplement programme freely offered at Government health centers. Though, Hb levels were low in the controls as well in those with and without HIV, clinicians have to monitor HIV patients for changes in Hb levels particularly while on HAART.

Red blood cell count and Packed cell volume

PCV is mainly contributed by RBC. Since all the groups had low RBC count, PCV was also low. PCV did not differ significantly between the groups.

Erythrocyte sedimentation rate

ESR was found to be significantly elevated (P<0.005) in the HIV+ TB+ group than the control group. Elevated ESR in HIV+TB+ patients have been reported from South Africa previously. This might result from inflammation, coexisting medical problems, increasing viral load, stage of disease, immune status, smoking habits, nutritional status, reduced PCV and other associated physiopathological mechanisms. Another study from Nigeria reported elevated ESR in all HIV positive patients, with about 95% having values more than 40 mm/hr. Similar observations was made in the present study also. Though ESR is elevated in the presence of infectious diseases, it is neither sensitive nor specific, and cannot be considered as general screening test.

A case-controlled study from Indonesia Karyadi, 18) reported significantly elevated erythrocyte sedimentation rate (82mm/hr) in pulmonary tuberculosis patients than in healthy controls (p<0.0001). This observation coincides with the present findings. A negative correlation was observed between ESR and Hb in all groups, as these patients had microcytic hypochromic anemia and contributed to the elevated ESR.

In summary, our findings reveal a positive association between mycobacterial and HIV infections with haematological abnormalities, and a synergistic effect of dual infection on the same. Socio-economic status might have also contributed to these changes. In view of the hematological alterations, practitioners should constantly monitor these cases and provide appropriate supportive measures.

Acknowledgment

Authors thank Dr.Luke Elisabeth Hanna, (Formerly TRC) NIRT, Chennai-31, Mrs. Rajeswari, Lab Technician, Government Rajaji Hospital, Madurai and DBT-BIF, at Department of the Biotechnology, Lady Doak College, Madurai Tamilnadu for providing technical help.

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