Autoimmune Haemolytic Anemia Associated With Some Diseases

Amany Mohammed Jasim and Safa A. Fadil
College of Medical & Health Technology, Baghdad

Received 24/4/2012 – Accepted 20/6/2012

ABSTRACT

The present study, extended from July 2011 to November 2011, a total of seventy two samples were collected from individuals their ages ranged from 16-70 years forty two was anaemic patients (16 males and 26 females) with chronic myelocytic leukemia (CML), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL) and rheumatoid arthritis (RA).

Other thirty samples were collected from non anaemic individuals as control group (10 males and females). All samples were tested by anti-human globulin (Coombs’ test), Preparation of blood film and Radial Immunodiffusion (RID).

It was observed that female more susceptible than male to the Auto immune hemolytic anemia (AIHA), no significant differences were noticed in the ages of AIHA patients compared with the control group. AIHA who gave positive coombs test (DAT), CML anemic patients had a higher AIHA percentage (47.62%), while HL patients had the lower percentage (4.76%). the incidence of +ve RH blood grouping of anemic patients with AIHA presented in this study was more susceptible to AIHA than –ve RH anemic patients. The distribution of blood grouping system was shown that blood group B (33.33%) was more susceptible than other groups. The results of precipitation reaction of IgG, IgM, IgA class and C3, C4 using immunodiffusion techniques. It was observed that the mean
result of C3 had a highly result of precipitation reaction among anemic patients with AIHA according to the normal averages of WHO (2003)

It also documented that autoimmune hemolytic anemia was strongly associated with increased risk of CLL among anemic patients studied.

INTRODUCTION

Hemolytic anemia is a condition of red blood cells destruction in which increased erythrocyte production is insufficient to keep up with accelerated RBC destruction, thus producing anemia [1].

It classified according to the mode of onset either inherited or acquire. Acquired hemolytic anemia may be classified as Alloimmune, drug induced autoimmune and other agents [2].

Autoimmune hemolytic anemia is characterized by increased red cell destruction and/or decreased red corpuscles survival due to autoantibodies directed against self-antigens on red corpuscles [3].

Autoimmune hemolytic anemia is classified to warm or cold hemolytic anemia based on clinical symptoms and on the optimal temperature [4].

The pathogenicity of AIHA due to deletion of self-reactive T-and B-lymphocytes clones during their maturation in the central lymphoid organs (the thymus for T cells and bone marrow for B cells [5].

Some people with AIHA may have no symptoms, other people developing symptoms like skin pale, weakness, chronic fatigue, shortness of breath dizziness, headache and rapid heartbeat [6].

Secondary AIHA are associated with some diseases including Chronic myeloid leukemia, Chronic lymphocytic leukemia, Acute lymphocytic leukemia, Non- Hodgkin lymphoma, Hodgkin lymphoma, Rheumatoid arthritis, systemic lupus erythematosus and viral infections [7].

Several factors including quantity and class of autoantibodies, complement activation affect in the ability of autoantibodies to provoke hemolysis, drugs and toxins may cause intravascular hemolysis through the formation of heinz bodies resulting in damaged membranes thus destroying RBCs [8].

The appropriate therapy of AIHA is dependent on the correct diagnosis and classification of this family of hemolytic disorders. Corticosteroid are standard, followed by consideration of splenectomy in complicated cases [9].

MATERIALS AND METHODS

The present study, extended from July 2011 to November 2011, a total of seventy two samples were collected from individuals their ages ranged from 16-70 years selected from Ebn AL-Balady hospital,
Baghdad Teaching Laboratories and National Centre for Research and Treatment of Hematology at Al-Mustansirya University Hospitals.

**Preparation of patient's samples:**

Five ml of peripheral venous blood sample were taken from forty two anaemic patients with CML, CLL, ALL, NHL, HL and RA (HB ≤ 8), none of these patients had received any treatment including blood transfusion during a period of three months, three ml were taken for Coomb's test and preparing blood film, sera were collected from the remaining blood sample for immunodiffusion technique. Thirty venous blood samples were collected from non anaemic individuals as control group.

1. **Anti-human globulin (Coomb’s test)**
   
   **Direct antiglobulin test (tube method)**
   
   This procedure was also carried out to anemic and non anemic patients according to [10].

2. **Radial Immunodiffusion (RID)**
   
   This procedure was carried out for anemic patients only to study a detailed serological characterization of autoantibodies of AIHA patients according to [11].

**Statistical analysis of data**

Parametric data were statistically analyzed using percentage and Fischer Exact Probability test at significant level P < 0.05 according to [12].

**RESULTS AND DISCUSSION**

Table (1) showed the distribution of studied patients with AIHA and control group by age and gender. It was observed that female were more susceptible than male to the AIHA. There was a significant increase in percentage of female than male patients at P < 0.05 using Fischer Exact Probability test. No significant differences were noticed in the ages of AIHA patients compared with the control group (AIHA patients age mean ± SE 42.88 ± 2.55, control group age mean ± SE 34.57 ± 2.8) using t-student.
Table-1: Distribution of studied patients with AIHA and control group by age and gender.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(years) AIHA patients</td>
<td>42.88 ± 2.55</td>
</tr>
<tr>
<td>Age(years) control group</td>
<td>34.57 ± 2.8</td>
</tr>
<tr>
<td>Female %</td>
<td>(61.9%)</td>
</tr>
<tr>
<td>Male %</td>
<td>(38.1%)</td>
</tr>
</tbody>
</table>

Figure(1) showed the number and percentage of anemic patients with AIHA who gave positive coombs test( DAT), CML anemic patients had higher AIHA percentage (47.62%) compared with other patients group, while HL patients had the lower percentage (4.76%) among all anemic patients studied.

![Figure-1: Number and percentage of anemic patients with AIHA positive DAT coombs test](image)

The most common laboratory findings associated with AIHA are found in figure (2,3,4) which represented by presence of spherocytes, and reticulocytes on the peripheral blood smear(fig.2,3) which reflect the immune mediated destruction of the erythrocyte, abnormalities of RBCs and rouleaux formation was also observed (fig.4) suggestive hemolytic process.
Figure-2: Blood smear of patients with ALL showing numerous spherocytes (Leishman stain x 40)  sp: spherocytes

Figure-3: Blood smear of patients with CML showing numerous reticulocytes (Leishman stain x 40)  R: reticulocytes
Figure-4: Blood smear of patients with NHL showing rouleaux formation (Leishman stain x 40) RO: Rouleaux

The distribution of blood grouping system and Rh among anemic patients with AIHA was shown in Table (2). It was observed that blood group B (33.33%) followed by O (28.57%) was more susceptible to the AIHA than AB (21.43%) and A blood group (16.67%)

Table-2: Distribution of blood grouping system and Rh among anemic patients with AIHA included in the work.

<table>
<thead>
<tr>
<th>Blood group system</th>
<th>No. %</th>
<th>Blood group and Rh</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood group B</td>
<td>14 33.33a</td>
<td>Blood group B+ve</td>
<td>11</td>
<td>26.19a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood group B-ve</td>
<td>3</td>
<td>7.14c</td>
</tr>
<tr>
<td>Blood group O</td>
<td>12 28.57a</td>
<td>Blood group O+ve</td>
<td>9</td>
<td>21.43a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood group O-ve</td>
<td>3</td>
<td>7.14c</td>
</tr>
<tr>
<td>Blood group AB</td>
<td>9 21.43b</td>
<td>Blood group AB+ve</td>
<td>7</td>
<td>16.67b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood group AB-ve</td>
<td>2</td>
<td>4.76c</td>
</tr>
<tr>
<td>Blood group A</td>
<td>7 16.67b</td>
<td>Blood group A+ve</td>
<td>6</td>
<td>14.29b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood group A-ve</td>
<td>1</td>
<td>2.38c</td>
</tr>
<tr>
<td>Total</td>
<td>42 100</td>
<td>Total</td>
<td>42</td>
<td>100</td>
</tr>
</tbody>
</table>

a, b, c Insignificant difference between similar litter using Fischer Exact Probability test at P < 0.01
Table-3: showed the mean results of precipitation reaction of IgG, IgM, IgA class and C3,C4 using immnodiffusion techniques. It was observed that the mean result of C3 had a highly result of precipitation reaction among anemic patients with AIHA according to the normal averages of WHO (2003)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IgG mg/dl N=39</th>
<th>IgM mg/dl N=41</th>
<th>IgA mg/dl N=39</th>
<th>C3 mg/dl N=34</th>
<th>C4 mg/dl N=34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1107.41 ± 381.81</td>
<td>112.51 ± 56.10</td>
<td>196.71 ± 81.57</td>
<td>614.21 ± 2196.81</td>
<td>36.81 ± 14.02</td>
</tr>
<tr>
<td>Normal (WHO)</td>
<td>710-1520</td>
<td>40-250</td>
<td>90-310</td>
<td>91-156</td>
<td>20-50</td>
</tr>
</tbody>
</table>

AIHA is a disease that causes decreased red cells survival due to abnormality of immune system to recognizing self–antigen and autoantibodies are made against RBCs antigen [13]. The present study found that the disease was not associated with age. Females most susceptible to the disease than males. This as may be due to stimulation of cells divisions by both estrogen and androgen hormones in females and elevated levels of these hormones may lead to abnormal cell growth such as autoimmune disease, ovarian cysts and lymphoproliferative disorders [14].

The present study had excluded all the patient samples who underwent blood transfusion processes to avoid a delayed transfusion reaction that may occur after 2-14 days blood transfusion and patients who has received chemical drugs and taken doses of radiation one months ago. Chemotherapy and radiation often also cause anemia, drugs could bind non specifically on the RBCs membrane of these patients that radiation formation of autoantibodies causing extravascular hemolysis destruction[15].

The present study demonstrated that autoimmune hemolytic anemia was strongly associated with increased risk of CLL. This finding agreed with the results of previous epidemiological study by [16].

[17] documented that warm AIHA are responsible for 70% of lymphoproliferative disorder such as CLL, NHL and HL [14]. Also noted strong associations between autoimmune hemolytic anemia and lymphoid neoplasms, even when a 5-year period before lymphoma diagnosis was excluded, suggesting that other mechanisms could be involved.

[18] documented that warm antibody hemolytic anemia is an autoimmune disorder characterized by the premature destruction of health red blood cells by autoantibodies. Other study by [19] mentioned that secondary AIHA was associated with viral infections, chemical agents, drugs and neoplastic diseases.

It was also shown that +ve RH blood grouping system was more susceptible to AIHA than –ve RH, this may be due to more specificity
reactive of autoantibodies against well defined RBCs antigens of +ve RH blood grouping system [19].

Microscopic finding of blood smears in present study showed numerous typical spherocytes and reticulocytes which provided a good indicator to hemolytic anemia. A defect in the surface membrane (the outer covering) of RBCs causes them to have a sphere, or ball-like, shape. These RBCs have life span that's shorter than normal [20]

[21] mentioned that the major cause of numerous spherocytes is warm-antibody hemolytic anemia in which the spleen removes small portions of the red cell membrane from cells coated with IgG destruction antibodies, erythrocyte then emerges from the spleen as a smaller cells.

[22] indicated that spherocytes are not as flexible as normal RBCs, and will be singled-out for destruction in the red pulp of the spleen as well as other portions of the reticuloendothelial system. The RBCs trapped in the spleen cause the spleen to enlarge, leading to the splenomegaly often seen in these patients.

The use of Immunodiffusion techniques could help to study the serological characterization of patients with AIHA with regarded to autoantibodies class and complement fractions. Demonstration of the antigenic serum components in patients with AIHA seemed to be associated with autoantibodies classes and complement fraction [23]

The present study revealed that several samples studied had the precipitation reactions either IgG or IgM and IgA and fixed complement, this was due to degree of hemolysis depending on characteristics of the bound antibodies (specificity, quantity and the ability to fix complement as well as the target antigen (density, expression and patients age)

[24] Our study showed that patients who had only C3 were in higher number among studied samples, fixing of complement caused intravascular hemolysis. As RBCs are warmed in the central organs, the bound immunoglobulines may be lost, leaving only bound C3 [24].

Reticuloendothelial cells also have receptors for complement factor present can potentiate the extravascular hemolysis [17].

Very few studies in Iraq on the hematological and serological evaluation of AIHA and other associated diseases were done therefore, more studies are requested to study the clinical evidences and the laboratory findings of such diseases.
REFERENCES


Autoimmune Haemolytic Anemia Associated With Some Diseases