Neutropenia Associated with X-Linked Agammaglobulinemia in an Iranian Referral Center

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ABSTRACT

X-linked Agammaglobulinemia (XLA) is a hereditary immunodeficiency, characterized by an early onset of recurrent bacterial infections, hypogammaglobulinemia and markedly reduced B lymphocytes number.

In order to determine the association of neutropenia among Iranian patients with XLA, hospital records of 30 patients with confirmed XLA in Children Medical Center Hospital, were reviewed.

Eight out of 30 XLA patients (26.7%) developed neutropenia during the course of the disease. In two patients, episodes of neutropenia were identified before or at the time of diagnosis of XLA. Other six patients whom were not visited regularly and did not receive periodical immunoglobulin replacement therapy experienced neutropenia after diagnosis of XLA.

Neutropenia in XLA is mainly associated with infection and is resolved with intravenous immunoglobulin replacement and antibiotics therapy.

Key words: Intravenous immunoglobulin; Neutropenia; X-linked agammaglobulinemia

INTRODUCTION

X-linked Agammaglobulinemia (XLA), a hereditary immunodeficiency, was first described in 1952.1 It is characterized by an early onset of recurrent bacterial infections, a profound deficiency of all immunoglobulin isotypes and a markedly reduced number of peripheral B lymphocytes.2,3 In 1993, the defective gene was cloned and called Bruto’s tyrosine kinase (BTK).4,5 The onset of recurrent bacterial infections typically occurs during the latter part of the first year of life, when maternal IgG, actively transported across the placenta, is reduced below the protective level. Over half of the patients with XLA are diagnosed by 2 years of age, and nearly 80% are diagnosed by school age.6,7 Intravenous immunoglobulin (IVIG) replacement therapy is the best choice and essential treatment for XLA, and effectiveness of intravenous immunoglobulin for reducing serious bacterial infections in XLA is well documented.8,9 BTK is expressed in B cell lineage and has a crucial in role in
Neutropenia Associated with XLA

early B cell development. Also, BTK is expressed in myeloid cells, monocytes, and platelets presumably participating in many myeloid cell functions.10-12

Although the determined defect in patients with XLA is related to early B cell defect and XLA is principally an antibody deficiency syndrome, some XLA patients might be associated with neutropenia. Based on different studies, the incidence of neutropenia among patients with XLA has been documented to be around of 10-26%.13-16

The purpose of this study was to determine the association of neutropenia among Iranian patients with XLA which had been diagnosed and treated in Children’s Medical Center Hospital.

PATIENTS AND METHODS

Among the XLA patients whom were registered in Iranian Primary Immunodeficiency Registry,17,18 the hospital records of 30 XLA patients had been diagnosed and treated in children medical center hospital affiliated to Tehran University of Medical Sciences were reviewed. The study was approved by the ethical committee of the university and informed consent was obtained from all patients.

Eight out of 30 patients who developed neutropenia are discussed in this paper. Diagnosis of XLA patients was made according to BTK gene mutation analysis (5 cases), defective expression of BTK using western blot method (2 cases) (Cell Signaling, USA),19,20 and profound hypogammaglobulinemia and markedly reduced peripheral B-cell (less than 0.1%) (one case). Serum immunoglobulin levels were measured by nephelometry and immunophenotyping was performed by flow cytometry. The mutated allele was detected by single-strand conformation polymorphism (SSCP) analysis.20

A Two-page questionnaire was designed to collect demographic data, age at diagnosis of XLA, age at diagnosis of neutropenia, number of episodes of neutropenia and clinical condition of at time of neutropenia. Neutropenia was defined as absolute neutrophil count (ANC) below than 1500/mm³. Neutropenia was considered mild, moderate and severe when ANC was between 1000-1500, 500-1000 and below 500/mm³, respectively.

RESULTS

In this study, the hospital records of 30 XLA patients with median age of 11 years (range: 1.5-28) were reviewed. The median levels of IgG, IgM and IgA were 130 (range: 10-420) mg/dl, 15 (range: 0–48) mg/dl, and 5 1 (range: 0–40) mg/d, respectively. In all cases, the number of peripheral circulating B cells was less than 1% of peripheral lymphocytes (Table 1).

Eight (26%) out of 30 patients developed at least one episode of neutropenia during the course of the disease (Table 1). Among our studied patients, one (P2) experienced 13 episodes of neutropenia, 4 patients (P1, P3, P4 and P6) had two episodes of neutropenia and 3 patients had one episode of neutropenia; totally 24 episodes of neutropenias were detected (Table 2). Except 1 patient (P6), occurrence of neutropenia in other patients was associated with bacterial infections, mainly respiratory tracts infections.

The diagnosis of XLA in these 8 patients was made at median age of 18.5 months (6-58 months), whereas occurrence of first episode of neutropenia was at median age of 31 months (10-53 months). Moreover, neutropenic episodes occurred after diagnosis in 7 patients, while 1 patient (P2) experienced 7 episodes before diagnosis and 6 episodes after diagnosis. Only in one patient (P2) episodes of neutropenia were recognized before diagnosis of XLA, and in 1 patient (P7) the diagnosis of XLA was made at the time of neutropenia, while other 6 patients experienced episodes of neutropenia after diagnosis of XLA. It should be noted that these 6 patients were not visited regularly in our clinic and did not receive regular IVIG.

The range of WBC counts in all 8 patients varied from 2200 to 8300/mm³. Among 24 detected episodes of neutropenia, ANC was less than 500/mm³ in 15 episodes (62.5%); ANC in 4 episodes was less than 100/mm³. In four episodes (16.66%) of neutropenia, ANC was between 500 and 1000/mm³ (moderate neutropenia), while mild neutropenia (ANC between 1000 and 1500/mm³) was identified in only 5 (20.83%) episodes (Table 1). One patient (P2) who had 13 episodes of neutropenia died due to pneumonia in his latest neutropenic episode with ANC of 118/mm³. Neutropenic episodes were associated with confirmed infections in skin, mucosa, pericardium, blood, ear and respiratory tract infection in 7 patients. Although bacterial infection was not found in one case (P5), further investigations revealed viral infection in this patient. Antibiotics in addition to IVIG were prescribed for all patients except P5 in whom only IVIG was indicated (Table 1). The duration of neutropenia in all patients were less than one week and resolved after treatment with antibiotic and IVIG therapy.
Table 1. Immunologic and mutation analysis characteristics in XLA patients with associated neutropenia.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>IgG (mg/dl)</th>
<th>IgA (mg/dl)</th>
<th>IgM (mg/dl)</th>
<th>B-cell (%)</th>
<th>Western Blot</th>
<th>BTK Domain</th>
<th>Mutation</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>Null</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>P2</td>
<td>100</td>
<td>10</td>
<td>20</td>
<td>0.8</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>P3</td>
<td>110</td>
<td>0</td>
<td>15</td>
<td>0.7</td>
<td>Null</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>P4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.06</td>
<td>Null</td>
<td>PH</td>
<td>349delA</td>
<td>N726fs.X120</td>
</tr>
<tr>
<td>P5</td>
<td>110</td>
<td>0</td>
<td>15</td>
<td>0.32</td>
<td>NA</td>
<td>SH3</td>
<td>895C&gt;T</td>
<td>R255X</td>
</tr>
<tr>
<td>P6</td>
<td>130</td>
<td>45</td>
<td>0</td>
<td>0.03</td>
<td>NA</td>
<td>SH3</td>
<td>895C&gt;T</td>
<td>R255X</td>
</tr>
<tr>
<td>P7</td>
<td>90</td>
<td>4</td>
<td>14</td>
<td>0.9</td>
<td>Null</td>
<td>PH</td>
<td>IVS1+5G&gt;C</td>
<td>Splice Donor Defect</td>
</tr>
<tr>
<td>P8</td>
<td>0</td>
<td>76</td>
<td>0</td>
<td>0.28</td>
<td>Pos</td>
<td>SH1</td>
<td>1783T&gt;C</td>
<td>Y551H</td>
</tr>
</tbody>
</table>

Table 2. Clinical and laboratory findings in XLA patients with associated neutropenia

<table>
<thead>
<tr>
<th>No.</th>
<th>Age of XLA onset</th>
<th>Age of XLA diagnosis</th>
<th>No. of neutropenia episodes</th>
<th>Age at first episode of neutropenia</th>
<th>WBC at lowest episode of neutropenia</th>
<th>Neutrophil (%)</th>
<th>ANC (µl⁻¹)</th>
<th>Associated Infection</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>3m</td>
<td>18m</td>
<td>2</td>
<td>53m</td>
<td>2200</td>
<td>5%</td>
<td>110</td>
<td>Stomatitis</td>
<td>Antibiotics+IVIG</td>
</tr>
<tr>
<td>P2</td>
<td>7m</td>
<td>19m</td>
<td>13</td>
<td>16m</td>
<td>5900</td>
<td>2%</td>
<td>118</td>
<td>Pneumonia+Diarrhea</td>
<td>Antibiotics+IVIG</td>
</tr>
<tr>
<td>P3</td>
<td>2m</td>
<td>14m</td>
<td>2</td>
<td>24m</td>
<td>8300</td>
<td>14%</td>
<td>1162</td>
<td>Tonsillo-pharyngitis</td>
<td>Antibiotics+IVIG</td>
</tr>
<tr>
<td>P4</td>
<td>34m</td>
<td>58m</td>
<td>2</td>
<td>72m</td>
<td>5300</td>
<td>1%</td>
<td>53</td>
<td>Pneumonia</td>
<td>Antibiotics+IVIG</td>
</tr>
<tr>
<td>P5</td>
<td>6m</td>
<td>6m</td>
<td>1</td>
<td>12m</td>
<td>5500</td>
<td>5%</td>
<td>275</td>
<td>No bacterial infection</td>
<td>IVIG</td>
</tr>
<tr>
<td>P6</td>
<td>18m</td>
<td>36m</td>
<td>2</td>
<td>38m</td>
<td>5200</td>
<td>4%</td>
<td>208</td>
<td>Pericarditis+Otitis Media</td>
<td>Antibiotics+IVIG</td>
</tr>
<tr>
<td>P7</td>
<td>10m</td>
<td>10m</td>
<td>1</td>
<td>10m</td>
<td>2790</td>
<td>25%</td>
<td>697</td>
<td>Sepsis</td>
<td>Antibiotics+IVIG</td>
</tr>
<tr>
<td>P8</td>
<td>20m</td>
<td>50m</td>
<td>1</td>
<td>42m</td>
<td>3200</td>
<td>28%</td>
<td>896</td>
<td>Cutaneous Pustules</td>
<td>Antibiotics+IVIG</td>
</tr>
</tbody>
</table>

DISCUSSION

This study documented that 26.7% of our investigated XLA cases were associated with neutropenia. In two previous studies performed by Lederman and Winkelstein\cite{14} and Plo Rodriguez \textit{et al.}\cite{16} the rate of neutropenia was reported 10% and 11%, respectively. Similarly, Kanegane \textit{et al.}\cite{13} showed that 16 of 87 patients with definitive diagnosis of XLA (18%) experienced neutropenia. The maximum rate of neutropenia was reported by Farrar \textit{et al.}\cite{15} who revealed that 13 of 50 confirmed XLA patients (26%) had neutropenia.

All these studies indicate that approximately 10-27% of XLA patients might be associated with neutropenia. The high rate of neutropenia in our study compared with other pervious reported rates is due to consideration of mild and moderate as well as severe neutropenia. If we included only severe neutropenia, the rate of associated neutropenia would be 16.7% which is still comparable to previous reports. The incidence of neutropenia in XLA might have been underestimated, since an episode of neutropenia may occur in the absence of severe infection or in a distant past might not have been registered in patient’s record.

Patients with XLA are mainly presented with infections; in a large multicenter cohort study of 201 patients with XLA, 170 patients (86%) initially presented with infection.\cite{7} Infections in this group of patients were sometimes associated with severe neutropenia.

All previous reports\cite{13,15,16} showed that neutropenia in XLA patient occurred before treatment with antibiotics and initiation of IVIG replacement therapy. Neutropenia in all reported patients resolved after adequate treatment supporting the role of infections inducing neutropenia. In contrast to pervious studies, in our study, 6 out 8 XLA patients developed neutropenia after diagnosis. The oc-
Neutropenia Associated with XLA

currence of neutropenia in these patients even after diag-

nosis could be due to irregular referring to IVIG clinic,
not receiving IVIG every 4 weeks, which consequently
result low serum IgG levels and increased susceptibility
to infections. Kozlowski and Evans postulated that
neutropenia in XLA patients could be the consequence of
destruction of neutrophils by endotoxin produced by
cacteria during severe infection.

Although BTK protein is expressed in myeloid cell
series, it is unclear whether BTK is required for neutro-

phil function or development of neutrophil cells during
the stress circumstances. However the etiology of neu-
tropenia in XLA patients is still unclear.

Neutropenia is also observed in other primary anti-

body deficiencies such as common variable immunode-

ficiency (CVID). Autoimmune mechanisms are the
main responsible causes of neutropenia, thrombocyto-

penia and hemolytic anemia in CVID patients. Neu-
tropenia also is the most common hematologic finding
occurring in over 60% of the patients with X-linked hy-

per IgM syndrome (XHIM). In patients with XHIM in
addition to autoimmune phenomenon suggested cause
for neutropenia it has been hypothesized that defect in
release of mature granulocytes from the marrow to the
peripheral blood may be a major cause of neutropenia in
XHIM. In contrast to CVID and XHIM, there is no
report regarding neutropenia induced by autoimmunity
in patients with XLA.

Kanegane et al. suggested that deficient cytokine or
chemokine production in monocytes could be the cause
of XLA-associated neutropenia; BTK is expressed in B-
cell development and monocytes; however, in BTK pro-
tein expression in monocytes of XLA patient is dimin-
ished. While phagocytosis and chemotaxis of monocyte
are reduced in XLA patients due to defects in BTK ex-
pression, LPS-induced TNF-a production is reduced in
monocytes of XLA patients and BTK may be engaged in
a "toll-like receptor pathway of monocytes". These
points led to that Kanegane et al. proposing probable
role of BTK in immunological response of monocytes in
XLA patients.

Although, XLA is an antibody deficiency, and re-
garding the high incidence of concurrent neutropenia,
especially in stress circumstances, physicians should
consider the superimposed effect of neutropenia that
may influence the clinical presentation as well as its
management.

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