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X – LINKED AGAMMAGLOBULINEMIA: CLINICAL AND IMMUNOLOGIC EVALUATION OF SIX PATIENTS*

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The clinical and immunologic features of six patients with X-linked agammaglobulinemia (XLA) are presented. The most common presenting manifestations were respiratory and gastrointestinal tract infections. On admittance to the hospital, one patient had a history of recurrent meningitis, another had a dermatomyositis-like syndrome, and still another had a history of recurrent arthritis. *Key words: agammaglobulinemia, Bruton's disease, T lymphocyte sub-populations.*

X-linked agammaglobulinemia (XLA) is a rare, sex-linked congenital immunodeficiency disease characterized by recurrent bacterial infections starting in infancy or early childhood, profoundly depressed serum immunoglobulins, a paucity of lymphoid tissue, and absence of B cells[1-3]. Pre-B lymphocytes are found in normal numbers in the bone marrow, peripheral blood or lymph nodes, suggesting a maturation defect in the differentiation of pre-B cells to B cells[4]. Cellular immunity is uniformly normal.

The aim of this report is to review the clinical and immunologic features and complications of XLA, and also to present the common and rare clinical presenting manifestations of this disorder.

Material and Methods

Six patients with XLA diagnosed between the ages of 20 months and six years were followed up in the Immunology Unit of the Hacettepe University Institute of

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Child Health. The diagnosis of each case was based on the following criteria of the World Health Organization for Immunodeficiency and XLA[5]. 1. Male sex, 2. Onset of disease in infancy or early childhood, 3. Paucity of lymphoid tissue, 4. Serum concentration of IgG less than 200 mg/dl and IgM and IgA concentrations less than 5 mg/dl, 5. Normal T cell numbers and normal proliferative response to T cell mitogens, 6. Very low number of B cells in the peripheral blood.

Delayed hypersensitivity reaction was evaluated by several antigens: Candida (prepared at the Hacettepe University Pediatric Allergy Labs) of a 1:10 dilution, phytohemagglutinin (PHA-P, Burroughs-Wellcome Labs) 10 μg/0.1 ml, PPD (Refik Saydam Hygiene Inst., Ankara) 5 U/0.1 ml, streptokinase-streptodornase (SKSD, Lederle Labs) 50/12.5 U/0.1 ml.

E-rosette forming cells were measured by the method of Jondal et al[6]. In vitro blastogenic response to PHA was studied according to a previously published method[7]. Serum immunoglobulins (IgG, IgM, IgA) were determined by using Behring-Werke immunodiffusion plates.

B cells were identified by direct immunofluorescence using commercial antisera against immunoglobulin isotypes (polyvalent, IgG, IgM, IgA; Behring-Werke Inst.)[8]. T lymphocyte subsets–pan T, helper T, and suppressor T–were determined by monoclonal antibodies (OKT3, OKT4, OKT8; Behring Werke Inst.) according to the standard indirect immunofluorescence technique[9].

Results

The clinical findings of our six patients are shown in Table I. Of the six males fulfilling the diagnostic criteria, only one patient (UYS) had a possible family history of the disease. His brother, who had recurrent infections beginning at the age of six months, died of meningitis at 13 months of age.

TABLE I: Clinical Features of XLA Patients

Case no.	Age at onset of symptoms	Age at diagnosis	Clinical manifestations at diagnosis	Present age
1.UYS	17 months	20 months	Dermatomyositis-like syndrome	6 years
2.AAR	9 months	22 months	Recurrent pneumonitis	19 years
3.OBC	10 months	2 years	Arthritis	7 years
4.EC	2 years	5 years	Recurrent meningitis	8 years
5.AO	4 years	6 years	Recurrent pneumonitis Chronic diarrhea Polio sequela	9 years
6.HB	1 year	5 years	Recurrent pneumonitis	exitus

The five remaining patients neither had male siblings nor symptoms of an immunodeficiency disease in their maternal uncles. Onset of symptoms was between the ages of 9 months to four years (mean age: 20 months). The mean age of the diagnosis of agammaglobulinemia was 3.6 years.

Infections were the major presenting feature. Three out of six patients had recurrent pulmonary infections. Patient UYS presented with a dermatomyositis-like syndrome and showed a dramatic response to gammaglobulin therapy, and patient EC had recurrent meningitis. The data of these two patients are published in other reports[10,11]. Recurrent arthritis was the presenting symptom in patient OBC.

Four patients had recurrent enteritis during the follow-up period and *Giardia lamblia* was isolated from the stools of these patients. One of these patients (OBC) developed malabsorption, hypoproteinemia, and edema due to giardiasis. Patient AO had hepatomegaly, and biopsy material revealed chronic active hepatitis. This patient had poliomyelitis two years prior to admittance to our hospital. During the follow-up period, patient AAR developed recurrent polyarthritis but no causative agent was identified from the synovial materials. The family of patient HB did not arrange for him to be given gammaglobulin regularly, and he died of pneumonia and sepsis. The immunological findings of our patients are shown in Table II. All the patients had a positive reaction to delayed hypersensitivity skin tests, and the percentages of E-rosette forming cells and in vitro blastogenic response to PHA were found to be within normal limits. Serum IgG levels were profoundly low (42-116 mg/dl),and IgA and IgM levels were undetectable. The percentage of Ig bearing cells (B cells) was very low (1-7 %). $CD3^+$ T cell percentages were above one standard deviation of normal levels in all

TABLE II: Immunologic Features of XLA Patients

Case No.	Delayed hypersensitivity skin test	Blastic transformation to PHA (SI)*	E-rosette formation** (%)	Serum immunoglobulins (mg/dl) IgG	IgM	IgA
1.UYS	+	42	72	42	0	0
2.AAR	+	57.4	68	144	0	0
3.OBC	+	59.9	81	92	0	0
4.EC	+	ND***	76	23	0	0
5.AO	+	31.7	65	46	0	0
6.HB	+	52	80	116	0	0

* Stimulation index (normal value for our lab: > 20)
** E-rosette formation (normal: 69 ± 5.6)
*** Not done

the patients (mean: 89±4.3) (Table III). $CD4^+$ T cells were found to have decreased in four patients and $CD8^+$ T cells to have increased in five. The helper/suppressor T cell ratio was found to be inverted in these five patients.

TABLE III: Lymphocyte Subsets of XLA Patients

Case No.	T Cell Subsets (%)				sIg⁺ (B) Lymphocytes (%)			
	$CD3^+$	$CD4^+$	$CD8^+$	CD4/CD8 ratio	sIg^+ polyvalent	$sIgG^+$	$sIgM^+$	$sIgA^+$
1.UYS	89	30	47	0.6	4	1	0	0
2.AAR	90	38	47	0.8	7	5	0	1
3.OBC	83	21	50	0.4	1	3	0	0
4.EC	95	54	27	2	2	0	0	0.5
5.AO	89	28	40	0.7	3	3	0	0
6.HB	82	25	49	0.5	4	2	2	1
Controls	66.9±13.3	45.6±5.2	27.0±6.9	1.8±0.5	22.2±5.0	9.1±3.6	8.2±4.2	4.5±3.1

Discussion

X-linked agammaglobulinemia (XLA) is a congenital antibody deficiency disease. Since Bruton's recognition of the first case of X-linked agammaglobulinemia in 1952, several terms (infantile panhypogammaglobulinemia, Bruton's disease, congenital agammaglobulinemia) have been used to describe this disease. Recently, the term "XLA" has been used to designate this entity[3]. Carrier detection can be done by using X-linked restriction fragment length polymorphism[12-14].

The histories and clinical presentations of patients with XLA vary. But respiratory and gastrointestinal tract infections, frequently in combination, are the most common presenting manifestations[1,2]. These symptoms were present in all our patients during the follow-up period. In the series of Lederman and Winkelstein[2], respiratory and intestinal infections were reported in 91 percent of the patients. These investigators indicated that infections in XLA are either recurrent or chronic, and that they occur on more than one site of the body and in combination. Gastrointestinal infections are the major presenting manifestations (35%) of the disorder. *Giardia lamblia* and viral pathogens (e.g. rotavirus) may cause chronic diarrhea[15,16]. We observed diarrhea in four of our patients and detected *Giardia lamblia* upon examination of the stool. Malabsorption, protein-losing enteropathy and disaccaridase deficiency have been described in agammaglobulinemic states[15,17,18]. In one of our patients (OBC), chronic diarrhea persisted for four weeks, and protein-losing malabsorption, hypoproteinemia and edema developed. Another one of our patients presented with recurrent meningitis, and another had a bout of meningitis at the time the diagnosis was made. It has been reported that meningitis occurred in 16 percent of patients in a larger series[2].

Patients with XLA are susceptible to chronic enterovirus infections[19-21]. Particularly chronic echo virus infections of the central nervous system have become evident, and often fatal, in patients with agammaglobulinemia. A dermatomyositis-like syndrome, a rare presenting manifestation, was seen in one of our patients. We observed muscle weakness in this patient, who responded dramatically to gamma globulin therapy. Electron microscopic examination of the muscle biopsy revealed virus-like particles. This clinical presentation might be a consequence of a systemic enterovirus infection[19].

Arthritis, which can be present during the clinical course of XLA, was observed at the follow-up of two of our six patients, and it was a presenting sign in another. No microorganism was identified from the synovial materials of these patients. Chronic middle ear infections were seen in four of the patients even after gamma globulin prophylaxis was initiated.

It has been reported that hearing loss due to chronic middle ear infection or a central nervous system infection occurs in approximately 32 percent of XLA patients[2]. Hearing loss was detected in one of our patients at the age of 15.

Rash, fever, anaphylactic-like reactions, and acrodynia have been described as complications of intramuscular gamma globulin therapy[1]. We observed a gamma globulin-induced anaphylactic reaction in only one of our patients.

During the follow-up period, hepatomegaly developed in one patient (AO), and, the biopsy material revealed chronic active hepatitis. This patient had poliomyelitis and developed sequela two years before admittance to our hospital.

In XLA, the common causes of death are cardiorespiratory failure as a consequence of recurrent pulmonary infections and severe viral infections[1-3]. All of our patients were diagnosed during early childhood, and gamma globulin treatment was given regularly, except for one patient. Chronic, obstructive lung disease and cor pulmonale were not observed during the patients clinical courses. One patient did not receive gamma globulin therapy regularly, and died of pneumonitis and sepsis.

In XLA, serum immunoglobulin concentrations are either very low or absent[22, 23]. In our patients, the serum concentration of IgG was very low, while serum IgA and IgM could not be detected. Lymphoid tissue is hypoplastic and lymph nodes do not have germinal centers. Peripheral B lymphocytes are absent or very low, and examination of the bone marrow reveals absent plasma cells which indicates that there is a differentiation defect between pre-B cells and B cells. This defect may not be complete and may be at more than one stage of maturation[24-27]. In all the patients, the number of surface immunoglobulin positive total B cells was markedly reduced. There were no surface IgM bearing lymphocytes in the peripheral blood in five of the patients and very few (2%) in one. Although

the number of IgM and IgD bearing lymphocytes in peripherial blood varies among patients, the percentage of circulating surface immunoglobulin positive B cells is always markedly low[24-27].

Cell-mediated immunity is intact in XLA. Although circulating T cells and T cell subsets are normal, minor alterations in the number and proportion of T cell subsets have been reported[28]. The alterations of T cell subsets and the $CD4^+$ / $CD8^+$ ratio, which we observed in our patients, might be related to recurrent infections or gamma globulin therapy.

The time period between the onset of symptoms and diagnosis has been reported to be (familial and non-familial forms) one year. In our series, it was 22 months. Since patients with XLA are susceptible to recurrent respiratory, gastro-intestinal and central nervous system infections, those patients presenting with these clinical manifestations should be immediately suspected of having this disease. Early diagnosis and treatment can prevent the development of early or late complications resulting from infections.

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