X-linked agammaglobulinemia caused by new mutation in *BTK* gene: A case report

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Aim. Primary immunodeficiencies (PID) are becoming a recognized public health problem worldwide. The most important subgroup of these disorders are the antibody deficiencies. X-linked agammaglobulinaemia was the first described entity of this group and is characterised by early onset of recurrent bacterial infections, profound deficiency of all immunoglobulin isotypes and markedly reduced number of peripheral B-lymphocytes.
Case report. We report the case of a 10-year old boy with X-linked agammaglobulinaemia caused by a previously non-described mutation in *BTK* gene with typical clinical presentation but delayed diagnosis. Following diagnosis, substitution therapy with intravenous immunoglobulins was started and the clinical status of the patient improved.
Conclusion. We reported a case of X-linked agammaglobulinaemia with delayed diagnosis despite the typical anamnestic signs for primary humoral immunodeficiency. The disease was caused by a previously non-reported mutation in the *BTK* gene. Measurement of serum immunoglobulins should be performed in all children with recurrent, complicated respiratory infections as a screening test for humoral immunodeficiencies.

Key words: awareness, B-lymphocytes, Bruton's tyrosine kinase, immunoglobulins, infectious complications, primary immunodeficiency, X-linked agammaglobulinemia

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INTRODUCTION

Primary immunodeficiencies (PID) encompass more than 250 disease entities of different incidence and clinical importance. Primary antibody (humoral) deficiency syndromes are a group of inherited disorders characterized by an inability to develop clinically effective immunoglobulin production. Nearly 75% of all primary immunodeficiencies are associated with defective immunoglobulin production. In the group of humoral immunodeficiencies are those with quite high prevalence, such as selective deficiency of IgA or transient hypogammaglobulinemia of infancy, but on the other hand, there are several syndromes with low prevalence and more severe clinical course. Those syndromes with quite high prevalence in the population are usually asymptomatic or have only mild clinical manifestation[1]. The most common clinical presentation of this PID group is recurrent infections, especially due to encapsulated bacteria, but there is also a variety of non-infectious complications such as autoimmune of lymphoproliferative disorders[2]. The first described disease from this group is X-linked agammaglobulinemia (XLA), which was previously named Bruton's agammaglobulinemia firstly described in 1952 (ref.[3]). Paediatric respiratory tract infections are one of the most common reasons for physician visits and hospitalisations and are associated with significant morbidity and mortality in childhood. The role of physicians and other healthcare providers is to discriminate between those with physiologically increased respiratory morbidity from the children suffering from severe pathological conditions leading to respiratory infections.

CASE REPORT

A 10-year old boy was transferred to our clinic due to complicated pneumonia with empyema. The boy was born as the 4th child at term with a birth weight of 3800 grams and length of 51 centimetres. Perinatal adaptation was unproblemmatic. From around 6 months of life, recurrent bacterial infections in various locations started. According to the documentation, the boy underwent several bacterial pneumonias, sinusitis with complication and recurrent otitis media. He had two attacks of acute pyelonephritis. Because of poor social conditions and lower hygiene standards, the diagnosis of ascaridosis, giardiasis and toxoplasmosis was also found in his history. Since the age of 3 years, he was regularly examined by a regional paediatric immune-allergologist for the suspected development of bronchial asthma. Despite recurrent and complicated bacterial infections, the concentration of serum immunoglobulins was not established. At the age of 9 years he suffered from purulent bacterial meningitis with consequent unilateral deafness. During the hospitalization at the Department of Infectology, significantly low immunoglobulins in serum were first detected. Further diagnostic algorithm and investigation were stopped due to

the missing consent of mother and poor compliance. One year after meningitis, he was admitted again to the hospital with the diagnosis of bilateral bronchopneumonia with left-sided pyothorax. Because of respiratory distress, pleural puncture was performed and the cultivation result of the pleural effusion showed *Streptococcus pneumoniae*. Owing to persisting changes on the chest X-ray and high inflammatory activity, the patient was transferred to the Intensive Care Unit of our Clinic for the surgical revision of left pleural cavity and re-drenaige (Fig. 1). CT of the lung revealed the encapsulated pyothorax of the left pleural cavity with pneumonia and subsequently pleurodesis with re-drenaige was performed. At the beginning of the stay at our Clinic, after the blood sampling for the needed immunological examination, the first infusion of intravenous immunoglobulins was applied. The pre-substitution results of immune parameters were consistent with the severe humoral immunodeficiency: IgG 0.20 g/L, IgA 0.03 g/L and IgM 0.11 g/L. In the peripheral blood, we did not detect the presence of $CD19^+$ B-lymphocytes. Taking into account the combination of severe hypogammaglobulinaemia of all three immunoglobulin isotypes, the absence of natural isohemaglutinins and $CD19^+$ B-lymphocytes together with male gender, the diagnosis of X-linked (Bruton) agammaglobulinaemia was suspected. The final diagnosis was confirmed by DNA molecular analysis. Genomic DNA was extracted from blood leukocytes according to standard protocols. Mutations were analyzed by amplifying exons 1–19 and the flanking intronic regions of the *BTK* gene by PCR, followed by sequencing with the Big Dye Terminator cycle sequencing kit on ABI3100 capillary sequencer (Life Technologies). The mutation c.504g>c was confirmed by restriction analysis with HpyCH4V after independent amplification of exon 5 and the same approach was used to identify mutation carriers. Molecular analysis of the causal gene for Bruton's tyrosine kinase (*BTK* gene) revealed the mutation in exone 5 in PH (pleckstrin homology) domain. This missense mutation p.W124C (g.51465G>C, p.Trp124Cys) leads to the changes of amino acid order in the protein with the subsequent changes in activity of BTK (at the level of DNA: substitution of guanine with cytosine, at the protein level: substitution of tryptophan with cysteine) (Table 1). This is a new, previously undescribed mutation leading to the development of XLA. The patient's mother is a carrier of this mutation but his brother does not carry the mutated allele. The patient is now substituted regularly with intravenous immunoglobulins with significant improvement in clinical status and decrease in infectious complications.

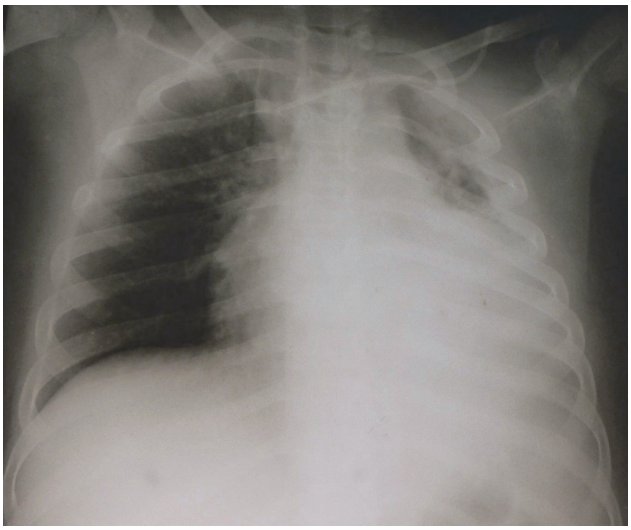

Fig. 1. Bilateral bronchopneumonia with left-side pyothorax in 10-year old boy with X-linked agammaglobulinaemia.

DISCUSSION

X-linked agammaglobulinaemia is one of the most clinically important deficiencies of humoral immunity. The disease is caused by mutation in the *BTK* gene (Xq21.3-Xq22), which encodes hematopoietic specific cytoplasmatic tyrosine kinase (Bruton's tyrosine kinase) expressed in different blood cells (except T-lymphocytes and terminally differentiated plasma cells).This molecule is extremely important in the signalling through B-cell receptor. The mutation of BTK leads to early halt in the development of B-lymphocytes. Approximately two thirds of the patients have familiar mutation, in one third new mutation can be found. Almost every family has its "private" mutation of BTK, and to date more than 450 different mutations (http://bioinf.uta.fi/BTKbase) have been identified (no single mutation accounts for more than 5% of patients). It is very important, that despite some general common symptoms of XLA, the genotype-phenotype correlation in XLA is weak[4]. According to the registry of the European Society of Immunodeficiencies, there are

Table 1. Description of the new mutation in *BTK* gene.

Genetic analysis of the *BTK* gene	
Affected gene	gene for Bruton's tyrosine kinase (Xq21.3-Xq22)
Mutation	Exone 5 → PH domain (pleckstrin homology domain)
Name of mutation	p.W124C (g.51465G>C, p.Trp124Cys)
Type of mutation	missense mutation → changes of amino acid order → changes in activity of BTK
Results of mutation	DNA: Δ guanine → cytosine Protein: Δ tryptophan → cysteine

7567 patients (55.2% of all registered patients) registered with predominantly antibody defects in Europe and 813 patients have agammaglobulinaemias (cca 80–85% of them with XLA). The main problem for this group of disorders was the diagnostic delay, but according to the latest analysis, there is a positive trend towards earlier diagnosis and appropriate treatment[5].

XLA is characterised by early onset of recurrent bacterial infections, profound deficiency of all immunoglobulin isotypes (with absent isohaemagglutinins and inability to produce specific antibodies), and markedly reduced number of peripheral B-lymphocytes but normal T-cell numbers and functions. On physical examination, paucity of lymphoid tissue can be found (small tonsils and lymph nodes). A significant number of patients remain asymptomatic in the first year of life and 80–90% are diagnosed during the first five years of life. Protection of children with XLA is performed within the first few months of life through the maternal antibodies which were actively transported through the placenta. With the on-going consumption of maternal antibodies the clinical manifestation with the recurrent infections becomes more evident. Recently it has been shown, that in addition to serum IgG levels, measurement of specific post-vaccination antibody levels may prove helpful in deciding when to begin IgG replacement in XLA infants[6].

Typical infectious complications are caused by pathogenic encapsulated bacteria (*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*), but typically these patients also suffer from giardiasis (lambliasis) and meningoencephalitis caused by echoviruses. In case of associated neutropenia, pseudomonas and staphylococcal sepsis can be present. Besides infectious symptoms, also non-infectious complication can be observed in XLA patients (e.g. idiopathic bowel diseases, seasonal allergies, aseptic arthritis, dermatomyositis-like syndrome, etc.) (ref.[7]). Similar to other X-linked disorders, XLA is lethal without medical intervention. The only effective treatment consists in regular substitution of immunoglobulins – intravenously or subcutaneously. Early treatment with the immunoglobulins shortly after the diagnosis leads to significant reduction in infectious symptoms. In specific cases, prophylactic antibiotic therapy too can be applied. It has been suggested, that B-cell-specific gene therapy could also be al treatment of XLA in the future[8]. Due to its severity, XLA is a candidate for prenatal screening in the mothers with an affected son or other relative in the maternal line (e.g. brother, uncle, nephew) (ref.[9]).

We present here the case of X-linked agammaglobulinemia caused by a new mutation in the causative *BTK* gene with typical clinical presentations which were found in the patient's history. There are two other mutations reported in the same position in the literature: p.W124C (g.51465G>T, c.372G>T, p.Trp124Cys) leading to the substitution of guanine by thymine in DNA and tryptophan with cysteine in protein and p.W124C – non-sense mutation leading to the formation of stop codon with premature end of DNA transcription[10,11].

CONCLUSION

Despite low incidence of primary immunodeficiency, it is necessary to increase public awareness with the aim of early diagnosis and the soonest appropriate treatment. The basic laboratory tests in patients with recurrent and complicated respiratory infections should also consist in measurement of serum immunoglobulins. All children with detected significantly reduced immunoglobulin concentrations should be therefore examined by a paediatric immunologist. Substitution therapy of immunoglobulins is essential for the significant reduction in infectious complications and for the substantial improvement in the prognosis of XLA patients.

ABBREVIATIONS

BTK, Bruton's tyrosine kinase; ESID, European Society for Immunodeficiencies; Ig, Immunoglobulin; PID, Primary immunodeficiency; XLA, X-linked agammaglobulinaemia.

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