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ACHROMOBACTER XYLOSOXIDANS BACTEREMIA IN A CHILD WITH X-LINKED AGAMMAGLOBULINEMIA.

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ABSTRACT

X-linked agammaglobulinaemia (XLA) is caused by mutation of the BTK gene. Early immunological screening is important in children with recurrent infection to avoid delay in diagnosis and treatment. A 9-year-old boy with delayed XLA diagnosis presented with a history of recurrent sinopulmonary infection since the age of 1 year old. He had a severe *Achromobacter xylosoxidans* bacteraemia at the age of 8 years that was successfully treated with intravenous antibiotics. Further immunological screening and genetic testing confirmed the diagnosis of XLA and he was treated with intravenous immunoglobulin replacement therapy. This case provides a valuable information on the diagnosis, treatment and management of XLA, emphasising the importance of early immunological investigations to avoid diagnostic delay.

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ABSTRACT

X-linked agammaglobulinaemia (XLA) is caused by mutation of the BTK gene. Early immunological screening is important in children with recurrent infection to avoid delay in diagnosis and treatment. A 9-year-old boy with delayed XLA diagnosis presented with a history of recurrent sinopulmonary infection since the age of 1 year old. He had a severe *Achromobacter xylosoxidans* bacteraemia at the age of 8 years that was successfully treated with intravenous antibiotics. Further immunological screening and genetic testing confirmed the diagnosis of XLA and he was treated with intravenous immunoglobulin replacement therapy. This case provides a valuable information on the diagnosis, treatment and management of XLA, emphasising the importance of early immunological investigations to avoid diagnostic delay.

Keyword: X-linked agammaglobulinemia, Bruton's Disease, *Achromobacter xylosoxidans*, Immunological screening.

INTRODUCTION

X-linked agammaglobulinaemia (XLA), otherwise known as Bruton's disease, is a primary immunodeficiency disease (PID) first described by Bruton in 1952. It is characterised by defective B cell maturation caused by mutation of the BTK gene that results in deficient antibody formation and defective antibody function.[1] XLA is a rare disorder that predominantly affects males because of the X-linked recessive inheritance pattern. Its clinical symptoms usually manifest between 6 months of life up to early adulthood, and it commonly presents with recurrent sino-pulmonary bacterial infection. Early immunological workup, such as serum immunoglobulin and lymphocyte subset enumeration in cases of recurrent infection, is important to avoid delay of diagnosis. Early replacement of immunoglobulin therapy decreases the rate of admission and

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morbidity for chronic complications, such as bronchiectasis and chronic lung disease.[2]

Herein, we describe a case that presented with *Achromobacter xylosoxidans* bacteremia, recurrent sino-pulmonary infection, and joint pain. This case emphasises the importance of early screening for primary immunodeficiency in children with recurrent bacterial infection.

CASE REPORT

The patient was a 9-year-old boy from a non-consanguineous family. He had a history of recurrent otitis media and upper respiratory tract infection since he was a year old, with more than four episodes per year requiring antibiotic treatment that resolved with multiple courses of antibiotics. On his 8th birthday, he developed a fever that lasted for 3 weeks and was associated with loss of appetite, loss of weight and lethargy. He was diagnosed with disseminated Gram-negative bacteraemia and serial blood cultures grew *Achromobacter xylosoxidans*, which was sensitive to ceftazidime. After 14 days of intravenous ceftazidime, he responded well and was discharged.

After 2 months, he presented again to our follow-up clinic with poor appetite, weight loss, productive cough and inability to ambulate due to left knee pain. Further examination at that time was suggestive of left knee arthritis. He had two episodes of febrile illness since his discharge that necessitated the use of oral antibiotics. He was treated as a case of bronchopneumonia, and further workup for primary immunodeficiency was performed in view of the history of repeated sino-pulmonary infections and isolation of *Achromobacter xylosoxidans* from blood cultures.

Physical examination of the patient revealed the absence of lymphoid tissue such as tonsil and lymph nodes. Initial serum immunoglobulin investigation revealed pan-hypogammaglobulinemia and the lymphocyte subset enumeration revealed the absence of B cells (Table I). There was an absent of BTK protein expression from flowcytometry examination (Figure 1). Targeted gene sequencing for PID panel was performed in a private laboratory in another country. Genetic analysis was positive for Bruton's tyrosine kinase (BTK) X-linked mutation on exon 10. c.862C>T. (p.Arg288Trp). This variant was previously reported in individuals with XLA.[3]

Intravenous immunoglobulin (IVIG) (0.4 g/kg) was started once every 3 weeks. After regular IVIG administration, his condition improved with lesser breakthrough infection and good weight gain.

DISCUSSION

The prevalence of XLA has been estimated to be about 1 case per 250,000 live births.[2] The prevalence of primary immunodeficiency diseases in Malaysia is 0.37 per 100,000 population; to date, 17 cases of XLA have been reported from Malaysia.[4] The numbers are extremely low compared with those in other registries published worldwide. Therefore, efforts to improve awareness about this disease must be aggressively pursued among the local medical fraternity.

Table I: List of immunological investigations result.

Immunological investigations	Result	Reference Range
Immunoglobulin G	0.75 (g/L)	2.6 – 15.2
Immunoglobulin A	<0.1 (g/L)	0.16 – 1.1
Immunoglobulin M	0.3 (g/L)	0.16 – 1.2
T cell	5790 (cells/UL)	1200-2600
T helper	1940 (cells/UL)	650-1500
T cytotoxic	3657 (cells/UL)	370-1100
B cell	0 (cells/UL)	270-860
NK cell	169 (cells/UL)	100-480

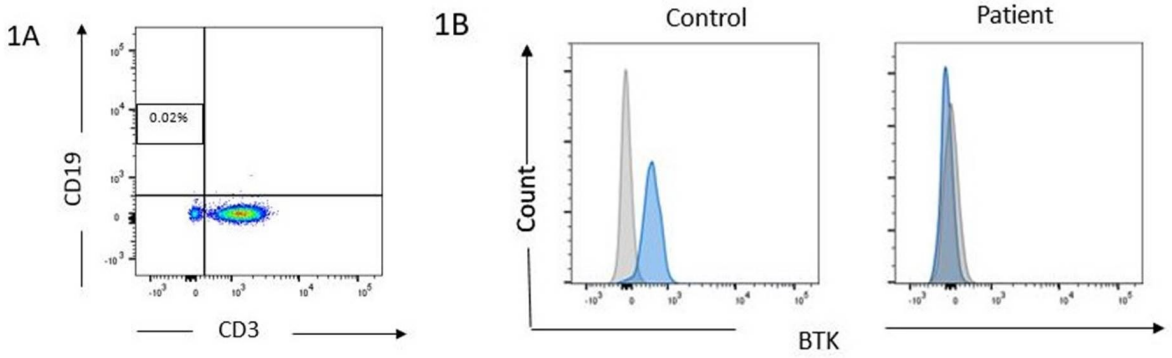

Figure 1: Flow cytometric analysis of Bruton's tyrosine kinase (BTK) expression in blood lymphocytes of this patient with X-linked agammaglobulinaemia (XLA). Mononuclear cells were stained with PerCP Cy5.5-labelled anti-CD3 and FITC-labelled anti-CD19 monoclonal antibody (MoAb). Intracellular BTK staining of monocytes was then performed by labelling peripheral blood with PE-labelled anti-CD14, followed by fixation, permeabilisation and labelling with Alexa Fluor 647-labelled anti-BTK or isotype control MoAb. Figure 1A, CD19+CD3- B cells are markedly reduced. Figure 1B, BTK expression in CD14+ monocytes are presented as a histogram. BTK expression is absent in the patient's monocytes. Grey-shaded area, isotype control; blue-shaded area, anti-BTK MoAb. (Click on image to enlarge)

The criteria for XLA diagnosis have been published by the Pan-American Group for Immunodeficiency and the European Society for Immunodeficiencies (Table II).[1] As per their guidelines, XLA diagnosis is made upon suggestive clinical manifestation associated with absent immunoglobulin levels, absent circulating B lymphocyte cells, absent BTK protein expression on monocytes and/or confirmed BTK gene mutation. About two-thirds of XLA cases are sporadic and arise from new genetic mutations.[5]

The clinical onset of XLA typically occurs after 6 months of age, when maternal immunoglobulin has been catabolised and the infant has become dependent on his own immune system. Our present case was diagnosed with XLA late at the age of 9 years compared with recent reports from Italy with overall mean age at diagnosis of 66 months.[6]

The majority of patients with XLA are not diagnosed with immunodeficiency until they are hospitalised due to infection. Our patient reported recurrent sino-pulmonary infections since the age of 1 year old. Notably, he was hospitalised for severe disseminated *Achromobacter xylosoxidans* bacteremia. The present case fulfilled the 10 warning signs for first line immunological screening.[7] These warning signs are an important measure to increase awareness of primary immunodeficiency among practitioners for early

Table II: Guidelines for the diagnosis of X-linked agammaglobulinaemia published by the Pan-American Group for Immunodeficiency and the European Society for Immunodeficiencies.[1]

Definitive	A male patient with less than 2% CD19 B lymphocytes and at least one of the following: 1) Mutation in BTK. 2) Absent BTK mRNA on northern blot analysis of neutrophils or monocytes. 3) Absent BTK protein in monocytes or platelets. Maternal cousins, uncles or nephews with less than 2% CD19 B lymphocytes.
Probable	A male patient with less than 2% CD19 B lymphocytes in whom all of the following are positive: 1) Onset of recurrent bacterial infections in the first 5 years of life. 2) Serum IgG, IgM and IgA more than 2SD below normal for age. 3) Absent isohemagglutinins and/or poor response to vaccines. 4) Other causes of hypogammaglobulinaemia and B lymphocytopaenia have been excluded.
Possible	A male patient with less than 2% CD19 B lymphocytes in whom other causes of hypogammaglobulinaemia and B lymphocytopaenia have been excluded and at least one of the following is positive: 1) Onset of recurrent bacterial infections in the first 5 years of life. 2) Serum IgG, IgM and IgA more than 2 SD below normal for age. 3) .Absent isohemagglutinins

immunological screening. The 10 warning signs are as follows: ≥4 ear infections in one year; ≥2 serious sinus infections in one year; ≥2 pneumonias in one year; recurrent, deep skin or organ abscesses; persistent thrush in mouth or fungal infection on skin; ≥2 deep-seated infections including septicaemia; ≥2 months on antibiotics with little effect; need for intravenous antibiotics to clear infections; failure of an infant to gain weight or grow normally and/or family history of primary immunodeficiency.[7]

The clinical manifestation of our case was similar to those previous published studies.[2,6] The most common clinical features were respiratory infection, with pneumonias, otitis media and sinusitis recorded in 39.9%, 32.7% 17.9% of the cases, respectively. A recent multicentre study from a developing country reported *Pseudomonas aeruginosa* followed by *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Klebsiella pneumoniae* as the common aetiology organism affecting XLA patients.[8]

Achromobacter xylosoxidans is an uncommon cause of infection in immunocompetent individuals. It is an aerobic, non-lactose fermenting gram-negative bacilli organism and can be found in water and soil (such as well water, swimming pool, dialysis solutions and chlorhexidine solutions).[9] It can cause a range of infections in those who are immunocompromised or immunodeficient mainly in the form of meningitis, sepsis and inflammation.[10] There have previously been published reports of XLA and other types of PID presenting with juvenile arthritis-like symptoms and *Achromobacter xylosoxidans* infection which is similar to this case presentation.[10-11] The treatment for *Achromobacter xylosoxidans* remain challenging as it has intrinsic and acquired resistant mechanism. The mainstay of treatment is guided by culture and sensitivity with initial therapy of anti-pseudomonal penicilines or carbapenems and removal of any identified locus of infection such as infected central venous catheter or peritoneal dialysis catheters.[12]

Early immunological investigations are the primary steps in patients presenting with recurrent sino-pulmonary infections. These investigations should involve quantification of lymphocyte subsets and serum immunoglobulin levels (IgG, IgM, IgA and IgG subclasses).[1,2] This measure enables identification of agammaglobulinaemia and may direct attention to early functional assay and genetic testing. Early diagnosis and prompt administration of intravenous immunoglobulin replacement therapy are important measures to prevent organ comorbidities.

Calculated globulin, which is measured as total protein minus albumin level, has been proposed as another potentially cheap and widely available screening test for antibody deficiency.[13] The proposed globulin cut-off value is 18g/L, which corresponds to an IgG level of 6 g/L, as the lower limit of normal range that warrants measurement of immunoglobulin levels. The serial calculated globulin of our patient was less than 18 g/L since the onset of sepsis and with low IgG levels. However, further prospective multicenter study is required to confirm the role of calculated globulin as a screening method for XLA.

The recent development of kappa-deleting recombination excision circles (KREC) as a potential newborn screening for primary antibody defect is still at a nascent stage and not available in Malaysia, and additional data are needed before it can be successfully implemented worldwide.[14]

CONCLUSION

Clinicians must have a high index of suspicion for the possibility of XLA diagnosis in patients who present with recurrent sino-pulmonary

infections. Early immunological investigations may hasten diagnosis for patients with XLA.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

This study has been approved by Human Ethical Research Committee, Universiti Sains Malaysia (JEPeM-USM). The approval number is USM/JEPeM/19080506.

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