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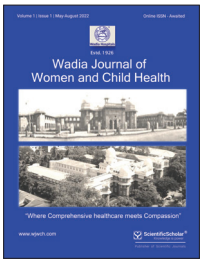

Case Report

Caspase-8 deficiency-a rare cause of immune dysregulation – First case report from India

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ABSTRACT

A 3½-year-old boy presented with generalized lymphadenopathy since 1 year of age. After 5 months of age, he had multiple infections and two episodes of pneumonia. At 2½ years, he developed chronic diarrhea. Colonoscopy showed ileal ulcers and inflammatory pan colitis with skip areas and histopathologic examination showed mild chronic ileitis, villous shortening, and diffused active colitis. Next-generation sequencing revealed a previously reported homozygous missense mutation Caspase-8. Unlike autoimmune lymphoproliferative syndrome, Caspase-8 deficiency states have immunodeficiency, autoimmunity, and early-onset inflammatory bowel disease in addition to lymphoproliferation. Early diagnosis aided by molecular confirmation is essential as haploidentical hematopoietic stem cell transplant is curative.

Keywords: Caspase-8, Caspase-8 deficiency state, Lymphoproliferation, Immune dysregulation

INTRODUCTION

Inborn errors of immunity are monogenic diseases with varied manifestations ranging from immunodeficiency to immune dysregulation. Disorders of immune dysregulation are associated with clinical features such as multiple autoimmunity, lymphoproliferation, and malignancies.[1] Disorders of immune dysregulation due to monogenic causes of impaired apoptosis are associated with recurrent and/or persistent lymphoproliferation.[2] Apoptosis is required for curbing the immune response by eliminating the activated T lymphocytes. There are two major apoptotic pathways, the death receptor (DR) pathway and the mitochondrial pathway, both culminate in the downstream activation of effector caspases. Caspases are proteases with cysteine residues in their catalytic site that selectively cleave proteins at sites just c-terminal to aspartate residues. Caspase-8 plays a critical role in apoptosis induced by various DRs such as CD95 (Fas), TNFR1, DR3, DR4, and DR5. The binding of the Fas ligand to Fas brings about recruitment of Fas-associated death domain (FADD), an adaptor molecule, to the receptor. FADD along with Fas and initiator procaspase-8 form a death-inducing signaling complex. Procaspase-8 is then cleaved to form activated Caspase-8. Activated Caspase-8 then activates the drown stream effector caspases 2, 3, 6, and 7 and also cleaves pro-apoptotic Bid, a member of the Bcl-2 family. The truncated

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Bid polypeptide then translocates to the mitochondria, leading to activation of the mitochondrial pathway by the release of cytochrome c [Figure 1].[3,4] Caspase-8 deficiency adversely affects T lymphocyte homeostasis, leading to lymphoaccumulation.[2,3] Here, we report the case of a small boy with persistent lymphadenopathy who was later diagnosed to have Caspase-8 deficiency. To the best of our knowledge, this is the first case report from India.

CASE DESCRIPTION

A 3½-year-old boy came to us with a history of generalized lymphadenopathy since 1 year of age. He was the only child of a 3rd degree consanguineous marriage with an uneventful birth history. His first hospital admission was at 5 months of age for fever with rash which was treated as bacterial sepsis. At 2 years of age in view of multiple episodes of febrile illness and persistent lymphadenopathy, a bone marrow aspiration was done which was normal. He had two episodes of radiologically proven lower respiratory tract infection around 2½ years of age; however, no causative organism was isolated. At 2½ years of age, he also developed chronic diarrhea which was investigated by 3 years of age. Stool examination showed the presence of leukocytes and stool calprotectin was not elevated. No organism was isolated from stool culture and staining for cryptosporidium was negative. USG abdomen was normal. Upper gastrointestinal tract endoscopy was normal. Histopathologic examination (HPE) of duodenal biopsy specimen was suggestive of chronic active duodenitis. A colonoscopy was done which showed ileal ulcers and inflammatory pan colitis with skip areas. HPE showed mild chronic ileitis with villous shortening and diffused active colitis.

He was referred to us suspecting an underlying immunological disorder at 3½ years of age with fever and acute gastroenteritis. On examination, he had failure to thrive, generalized significant lymphadenopathy involving bilateral cervical lymph nodes, axillary, and inguinal lymph nodes with hepatosplenomegaly. Laboratory investigations revealed microcytic hypochromic anemia, leukocytosis, and thrombocytosis. C-reactive protein was elevated and stool microscopy showed 25–28 leucocytes per high-power field. Stool culture grew *Klebsiella pneumoniae*. He received intravenous antibiotics based on the sensitivity pattern. We investigated him for an underlying primary immunodeficiency. Lymphocyte subset analysis showed T-cell lymphocytosis (Absolute CD3+/CD19− T lymphocytes – 10,804/mm^3 and normal range – 1400–7000/mm^3) with and inversion of CD4/CD8 ratio (CD3+CD4+ Helper T-cells – 2431/mm^3, normal range – 700–2200/mm^3; CD3+CD8+ Cytotoxic T-cells – 7698/mm^3, normal range – 490–1300/mm^3) [Table 1]. The percentage of double negative T-cells (DNT) was normal (0.8%) which ruled out autoimmune lymphoproliferative syndrome (ALPS). Human leucocyte antigen (HLA-DR) expression on T-cells was marginally

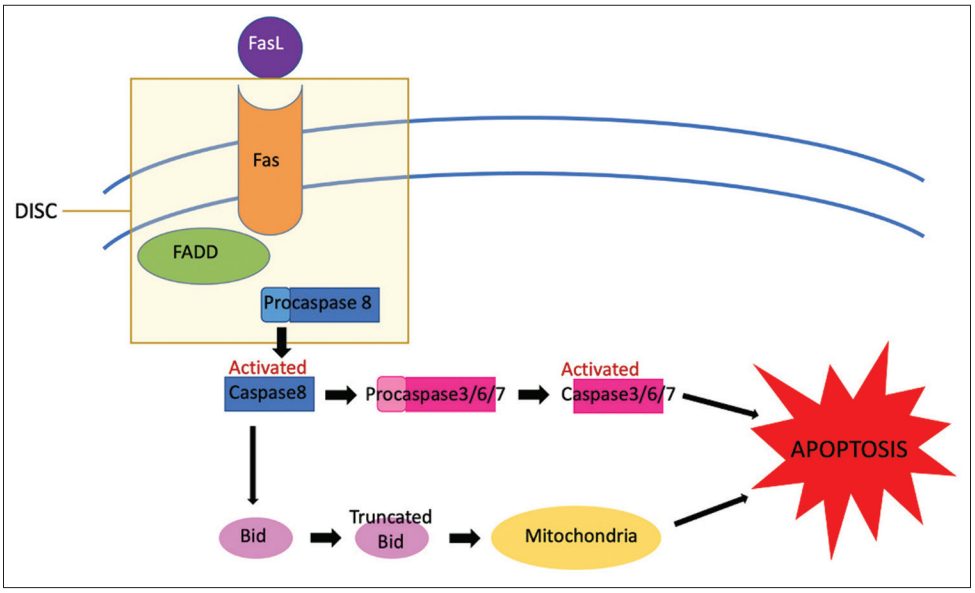

Figure 1: Apoptotic pathway down-stream of Fas. The binding of Fas ligand to Fas brings about recruitment of Fas-associated death domain (FADD), an adaptor molecule, to the receptor. FADD along with Fas and initiator procaspase 8 form death induced signaling complex (DISC). Procaspase 8 is then cleaved to form activated Caspase 8. Activated Caspase 8 then activates the drown stream effector caspases 2, 3, 6, and 7 and also cleaves pro-apoptotic Bid, a member of the Bcl-2 family. The truncated Bid polypeptide then translocates to the mitochondria leading to activation of the mitochondrial pathway by release of cytochrome c.

Table 1: Findings of lymphocyte subset analysis and immunoglobulin levels done at our center at first visit.

Parameter	Value	Normal range
Absolute lymphocyte count	13,505/mm^3	2300–5400/mm^3
Absolute CD19+B lymphocytes	1351/mm^3	390–1400/mm^3
Absolute CD3+/CD19−T lymphocytes	10,804/mm^3	1400–3700/mm^3
Absolute CD3+/CD4+T helper lymphocytes	2431/mm^3	700–2200/mm^3
Absolute CD3+/CD8+T cytotoxic lymphocytes	7698/mm^3	490–1300/mm^3
CD3-/CD16+56+NK cells	1215/mm^3	130–720/mm^3
Immunoglobulin G	1700 mg/dL	345–1236 mg/dL
Immunoglobulin A	181 mg/dL	14–159 mg/dL
Immunoglobulin M	126 mg/dL	43–207 mg/dL

elevated (HLA-DR – 34% on T-cells). Furthermore, CD19 + Absolute B lymphocytes were within normal limits (1351/mm^3, normal range – 390–1400/mm^3). NK cells were also elevated (1215/mm^3, normal range – 130–720/mm^3). Immunoglobulin M (IgM) levels were normal (126 mg/dL, normal range – 19–146 mg/dL) IgG (1700 mg/dL, normal range – 453–916 mg/dL) all the immunoglobulin reference ranges are different from table and IgA (181 mg/dL, normal range – 20–100) were elevated.

Next-generation sequencing revealed a homozygous, autosomal recessive missense mutation in exon 7 of Caspase-8 gene at chromosome position chr2:g.202141631C>T. The transcript found in our patient was c.919C>T leading to protein change p.Arg307Trp. This variant has been reported as deleterious by SIFT and probably damaging by PolyPhen. The same mutation, chr2:g.202141631C>T, also gives rise to transcript c.742C>T, leading to protein change p.Arg248Trp which has been previously reported by Chun *et al*[5] and Niemela *et al*[8] in patients with Caspase-8 deficiency. The child underwent a haploidentical hematopoietic stem cell transplant (HSCT), the donor being his father. He is doing well at 3 years of follow-up.

DISCUSSION

Homozygous mutations in Caspase-8 gene are associated with defective lymphocyte apoptosis. In contrast to patients with ALPS, they also have hampered activation of T and B lymphocytes and NK cells leading to immunodeficiency.[5] Caspase-8 acts as a molecular switch that controls necroptosis and pyroptosis.[6] It's deficiency is associated with increased NLRP3 inflammasome activity in the macrophages and increased production of proinflammatory cytokine interleukin-1 beta which is responsible for inflammatory phenotype.[7] Homozygous mutations in Caspase-8 were previously considered as ALPS type IIb. However, as these patients have immunodeficiency in addition to lymphoproliferation, Caspase-8 deficiency is now considered as a separate entity termed as "Caspase-8 deficiency state" (CEDS).[2]

The first two cases of CEDS were reported in 2002. These two children were siblings with persistent lymphadenopathy, splenomegaly, and recurrent sinopulmonary infections, features seen in our patient as well.[5] Susceptibility to Epstein-Barr virus, Herpes simplex virus infections, has also been demonstrated, but was not observed in our patient.[5,8,9] Recurrent infections have been suggested to be due to defective innate immune response and due to the role of Caspase 8 in activation of nuclear factor Kappa B downstream of T-cell receptor leading to T-cell defect.[8,9] The association of CEDS with gastrointestinal involvement in the form of chronic diarrhea and early-onset inflammatory bowel disease, as seen in our patient, has also been demonstrated in previous studies.[5,7,8] The loss of catalytic activity of Caspase 8 in the intestinal epithelial cells is responsible for the exaggerated inflammation as has been demonstrated in mouse models and was seen in the HPE of our patient's endoscopic biopsy.[6] Other autoimmune manifestations such as autoimmune hemolytic anemia and autoimmune thyroid disease have also been reported in patients with CEDS.[2] Our patient did not have any other autoimmune manifestations.

T-cell immunophenotyping in our patient showed CD4+ and CD8+ lymphocytosis. As seen in the study by Niemela *et al*, our patient too had an inversion of CD4/CD8 ratio. Kanderova *et al* demonstrated increased activated T-cell population with reduced naïve cells with a maturation shift toward CD4+ and CD8+ effector memory cells.[8] The expression of HLA-DR, an activation marker, on our patient's T-cells, was marginally elevated. T-cell lymphocytosis is also seen in patients with ALPS; however, they have elevated double negative T-cells (elevated DNT is considered as ≥1.5% of total lymphocytes or ≥2.5% of CD3+ lymphocytes).[10] As seen in most other CEDS, our patient too had a normal percentage of DNT cells, thus ruling out ALPS.[5,8,9] Our patient had normal B and NK cell numbers in contrast to the previously reported B-cell lymphopenia.[8,9] All the previously reported cases have showed defective T-cell activation, proliferation, and apoptosis. However, these assays could not be performed in our patient.[5,7-9] Kanderova *et al* demonstrated dysgammaglobulinemia with reduced IgM and IgA levels with elevated IgG levels, whereas Niemela *et al* demonstrated hypogammaglobulinemia in their patients with Caspase-8 deficiency.[8,9] Our case had low IgM levels with elevated IgG levels.

CONCLUSION

CEDS is clinically characterized by recurrent infections, lymphoproliferation, and gastrointestinal involvement. The persistent lymphadenopathy has been suggested to be due to

chronic activation and accumulation of apoptosis resistant T-cells as against the accumulation of double negative T-cells as seen in ALPS.[8] Thus, all cases of suspected ALPS, especially those with immunodeficiency and/or inflammatory bowel disease, require a genetic work up to rule out CEDS. Early diagnosis is imperative as HSCT is curative and helps reduce the morbidity and mortality associated with this disease.

Ethics approval

Ethics approval was taken from the Institutional Ethics Committee, B. J. Wadia Hospital for Children, under the Center of excellence project by ICMR. Project number-IEC-BJWHC/AP/2014/003.

Helsinki Declaration was followed.

Acknowledgments

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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