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Rare Case Report: Clinical and Immunological Insights into ZAP-70 Deficiency

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1. Abstract

ZAP-70 deficiency is a rare primary immunodeficiency disorder characterized by a severe defect in T-cell receptor signaling, leading to impaired immune function. Early diagnosis and intervention play a crucial role in preventing life-threatening infections and improving patient outcomes. We presented a case involving a novel mutation in the ZAP 70 gene, presented with Burkholderia cepacia, an uncommon pathogen. Immunological assessment guided genetic testing, which confirmed the diagnosis through the identification of a novel homozygous missense mutation in the ZP 70 gene.

2. Keywords:
Zap70 deficiency, SCID, Combined Immunodeficiency, CD 8 lymphopenia

3. Background

ZAP-70 deficiency is a rare autosomal recessive primary immunodeficiency disorder that leads to severe combined immunodeficiency (SCID) due to impaired T-cell receptor (TCR) signaling. [1] Diagnosing ZAP-70 deficiency can be challenging because of its rarity and the overlap of its clinical features with those of other immunodeficiencies. [2] Here, we present a case to raise awareness among clinicians about this rare immunodeficiency, especially when it presents with atypical manifestations, to facilitate early diagnosis and improve survival outcomes. Informed consent was obtained from the patient's guardians, and ethical approval was secured from the institutional review board (IRB) for the publication of this case report. All procedures followed the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000.

A 7-month-old male, born of a third-degree consanguineous marriage, presented with symptoms of loose stools, fever, and severe dehydration. Physical examination also revealed lymphadenopathy. Blood culture identified Burkholderiacepacia, an uncommon pathogen. Laboratory evaluations showed normal serum immunoglobulin levels and a normal absolute lymphocyte count (ALC- 7,638/µl). Lymphocyte subset analysis revealed a normal distribution of T, B, and NK cells, but there was a markedly reduced number of cytotoxic T cells (Tc = 0.5%; absolute Tc count = 40/µl). Further immunological analysis revealed reduced levels of naive T cells, as well as memory and class-switched memory B cells, compared to age-matched healthy controls. Intracellular staining revealed abnormal ZAP-70 protein expression in T and NK cells. Functional assessment of the TCR signaling pathway, via stimulation with anti-CD3/CD28 antibodies and phytohemagglutinin (PHA), showed abnormal results when compared to healthy controls. Whole exome next-generation sequencing revealed novel homozygous missense R557W mutation in the ZAP-70 gene. This mutation was verified as pathogenic using established genetic databases and prediction software. The patient was started on prophylactic antimicrobial therapy to prevent opportunistic infections, and intravenous immunoglobulin (IVIG) therapy was administered to support humoral immunity pending restoration of T-cell function. The patient is currently awaiting hematopoietic stem cell transplantation (HSCT).

4. Discussion

ZAP-70 deficiency, a severe form of SCID, typically manifests within the first year of life, with a median onset at around 4 months. Parental consanguinity and a relevant family history are key indicators of this condition. Patients often present with recurrent respiratory and gastrointestinal infections, chronic diarrhea, failure to thrive, and sometimes autoimmune symptoms. Viral infections, especially CMV and Varicella, are the most common, followed by bacterial, fungal, and protozoal infections. Complications from BCG vaccination, infectious skin conditions, fungal abscesses, and serious viral infections like VZV encephalitis are also reported. [3]. Typical SCID presents with severe lymphopenia, but in ZAP-70 deficiency, patients often have normal or near-normal lymphocyte counts. A key feature is a low number of cytotoxic T cells (Tc cells), although elevated counts have been reported. ZAP-70 deficiency impairs TCR signal transduction, hindering the differentiation

of CD4+CD8+ double-positive T cells into mature CD8+ single-positive T cells. In ZAP-70 deficiency total T-cell counts may be normal or slightly reduced, often associated with reduced proportion of naive Th and Tc cells. Additionally, B and NK cells may be diminished. The present case shows normal counts of T, B, and NK cells with Tc cells lymphopenia. In SCID patients, the levels of naive Th and Tc cells levels are generally very low. However, in cases of ZAP-70 deficiency, the levels of naive Th cells may only be slightly reduced or fall within borderline levels. [4]

The reduced responses to PHA and anti-CD3/CD28 suggest potential Th cell dysfunction, despite their normal frequencies. [5] In our case, we observed that in healthy controls, T cells typically downregulate CD3 expression following activation with PHA and anti-CD3/CD28. However, in ZAP-70 deficient T cells, CD3 expression persists despite activation. This CD3 downregulation in response to CD28/CD3 and PHA stimulation is dependent on ZAP70 activation. ZAP70, a critical kinase in T-cell receptor (TCR) signalling, initiates the internalization of the receptor, reducing CD3 surface expression. In ZAP70-deficient patients, this signalling pathway is disrupted, preventing CD3 internalization and leading to CD3 retention on the cell surface even after stimulation. In general, Th cells of ZAP-70 deficiency patients demonstrate normal proliferation when stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin, which bypass TCR signalling and confirm an intact response to mitogenic stimuli. [6] This test was not performed in our case. Additionally, the reduced levels of memory and class-switched B cells support the hypothesis of non-functional Th cells. Estimating ZAP-70 protein levels on T and NK cells using flowcytometry offers a rapid phenotypic diagnosis for ZAP-70 deficiency. [7] Clinical and immunological assessments provide clues about the underlying genetic defect, which can be confirmed through whole exome sequencing by identifying novel missense mutations in ZAP-70 deficiency. [8]

The ZAP70 gene, located on chromosome 2, encodes the ζ-chain-associated protein kinase 70k Da (ZAP70), essential for T-cell development and function.[9] The stability of this protein is governed by a conserved Mx(2)CWx(6)R motif. Mutations in this motif, including M572L, W576L, and R583A, can lead to reduced or absent protein expression, thereby confirming ZAP70 deficiency. Disease-causing mutations in ZAP-70 are spread across the gene, mainly affecting the kinase domain, as well as other domains such as N-SH2, me-A, C-SH2, and me-B. A novel homozygous missense R557W mutation was identified in our case, which is in the kinase domain, linked to absence of Zap70 expression results in ZAP70 deficiency. [10] The Zap70 protein tyrosine kinase is involved in various cellular processes, including T-cell receptor signalling, positive and negative selection of thymocytes, and alternative antigen receptor signaling in T cells. It can also associate with other proteins, such as proto-Vav and SH2 domain-containing proteins, to modulate downstream signalling pathways. These findings underscore the critical role of Zap70 in immune function and highlight its potential as a target for therapeutic interventions in immunodeficiency disorders. [10] Supportive care, including prophylactic antibiotics and intravenous immunoglobulin (IVIG) therapy, is essential to manage infections and support the immune system until HSCT is performed. Post-transplant care is equally critical to monitor for complications such as graft-versus-host disease (GVHD) and to ensure successful immune reconstitution. The patient's positive outcome post-HSCT emphasizes the effectiveness of this approach when diagnosed early and treated promptly. [11]

The understanding of ZAP-70 deficiency has improved significantly with advancements in genetic testing and immunological assays. However, challenges remain in early diagnosis and treatment. Newborn screening for severe combined immunodeficiencies may facilitate earlier detection of ZAP-70 deficiency, allowing for prompt intervention before severe infections occur. In ZAP-70 deficiency, T-cell receptor excision circles (TREC) levels are typically low or absent due to impaired thymic output of naive T cells. As a result, newborn screening using TREC assays may flag ZAP-70 deficiency, allowing for early detection. However, because some cases may still have near-normal T-cell counts, the condition might occasionally evade detection through standard TREC-based newborn screening. [12] Future research could focus on developing gene therapy approaches to correct ZAP-70 mutations, offering a potential alternative to HSCT. Gene therapy could provide a targeted and less invasive treatment option, particularly for patients without suitable donors for HSCT. Additionally, exploring the role of ZAP-70 in autoimmune manifestations and how it influences T-cell tolerance could provide insights into the broader implications of ZAP-70 deficiency beyond immunodeficiency.

5. Conclusion

This case emphasizes the need to consider ZAP-70 deficiency in patients accompanied by the isolation of the rare pathogen Burkholderiacepacia. It highlights the diverse clinical presentations associated with ZAP-70 deficiency. The discovery Tc cell lymphopenia along with non-functional T cells, despite normal T cell counts, was a key indicator that led to genetic testing. Early identification of ZAP-70 deficiency through comprehensive immunological and genetic evaluation is crucial for initiating timely treatment, preventing severe infections, and improving patient prognosis.

6. Learning Points/Take Home Messages

- This case underscores the importance of recognizing infections with uncommon pathogens.
- The findings advocate for a comprehensive diagnostic approach, including advanced genetic testing, to identify rare mutations.
- Early identification and intervention can significantly improve outcomes and prevent severe complications in patients with primary immunodeficiencies like ZAP-70 deficiency

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