Use of phosphoSTAT1 as an alternative to interferon-stimulated gene expression to evaluate type 1 interferonopathies

AUTHORS

Asma Shaikh[1], Nidhi Desai[1] , Umair Bargir[1], Dr. Shilpa Kulkarni[2] , Dr. P. Sindhu[2] , Dr. Prashant H[3],Dr Ambreen[3] , Dr. Punit Jain[4] , Dr. Sarita Verma[5], Dr. Kanan Subramanian[5], Manisha Madkaikar[1].

Affiliations

1 Department of Pediatric Immunology and Leukocyte Biology, ICMR- NATIONAL INSTITUTE OF IMMUNOHAEMATOLOGY, KEM Hospital, Parel- Mumbai.

2 Department of Neurology, Bai Jerbai Wadia Hospital for Children**,** Parel- Mumbai.

3 Department of BMT, Bai Jerbai Wadia Hospital for Children**,** Parel- Mumbai.

4 Department of Haematology, Apollo Hospitals, Belapur, Navi Mumbai

5 Department of Haematology**,** KEM Hospital, Pune

ASMA A. SHAIKH
MSc- Senior Project Assistant
Department of Pediatric Immunology and Leukocyte Biology, ICMR- NATIONAL INSTITUTE OF IMMUNOHAEMATOLOGY, KEM Hospital.
Parel- Mumbai, 400012
Contact no- 9987311524
Email ID- asmashk21@gmail.com

Dr Manisha Madkaikar
Director
Department of Pediatric Immunology and Leukocyte Biology, ICMR- NATIONAL INSTITUTE OF IMMUNOHAEMATOLOGY, KEM Hospital.
Parel- Mumbai, 400012
Contact no- 9892472552
Email ID - madkaikarmanisha@gmail.com

BACKGROUND

Interferonopathies are autoinflammatory and autoimmune disorders characterized by excessive production or signaling of type I interferons. Interferon-stimulated gene expression (ISG) is often used to diagnose and monitor disease activity in interferonopathies. Here, we assessed STAT1 phosphorylation by flow cytometry as an alternative technology to evaluate interferon signaling in monogenic interferonopathies.

METHOD

5 patients with genetically diagnosed interferonopathies were enrolled in this study. ISG expression and phospho-STAT1 levels were measured concurrently. Whole blood collected in heparin-anticoagulated tubes was used to measure STAT1 phosphorylation at baseline and in response to

stimulation by IFN-α (type 1 interferon) and IFN-ɣ (type 2 interferon). Blood was collected in Tempus tubes to assess ISG score and STAT1 gene expression to preserve gene expression. Relative mRNA expression was measured by qPCR using SYBR green. ISG score was calculated as the median fold change in the normalized expression of 7 interferon-response genes (IFIT1, IFI27, MX1, SIGLEC1, RSAD2, ISG15, IFI44L) compared to healthy controls. Cut-off was set at 2.15 calculated as median+2SD from a pool of healthy controls.

RESULTS

ISG was elevated in all patients, irrespective of disease activity and ongoing medications. ISG in patients ranged from (7.80-85.11). Additionally, STAT1 was consistently overexpressed in patients, with an average 2.5-fold increase compared to controls. Despite a positive ISG score and increased expression of STAT1, none of the patients tested had basal phosphorylation of STAT1. However, upon stimulation with IFN-α and IFN-ɣ, the median MFI of STAT1 in patients was 15556 and 17903, respectively, compared to same-day controls, with median MFI of 7851 and 7111.

CONCLUSION

Phospho-STAT1 by flow cytometry may serve as a sensitive marker for interferon signaling. It may serve as an additional functional tool to validate variants of uncertain significance found in patients with monogenic disorders.