

T_H17 Cells in *STAT3* Related Hyper-IgE Syndrome

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Abstract

Objectives To assess the utility of T helper17 (T_H17) cell enumeration *vis-à-vis* National Institutes of Health (NIH) scoring in Hyper IgE syndrome (HIES).

Methods Clinical phenotypes of Hyper IgE syndrome patients with and without *STAT3* mutation were analysed and correlated with absolute eosinophil count, serum IgE levels and T_H17 cell numbers in 19 patients with clinically suspected HIES and compared with healthy controls (*n* = 20).

Results The difference in serum IgE between patients with and without *STAT3* mutation and healthy controls was statistically significant (*p* < 0.05). Six patients had NIH score > 40; of which 4 were positive for *STAT3* pathogenic variants, whereas two patients in the group with no identifiable *STAT3* pathogenic variant had NIH score > 40. NIH score had sensitivity of 80 % and specificity of 87.5 % to detect cases with *STAT3* pathogenic variants. T_H17 cells were markedly low in all cases with *STAT3* pathogenic variants. Among patients without *STAT3* pathogenic variants, none had low T_H17 cell numbers.

Conclusions Low T_H17 cell numbers together with NIH scores can be a better indicator for presence of *STAT3* mutations.

Keywords Hyper-IgE syndrome · NIH scoring · *STAT3* · T_H17 cells

Introduction

Hyper-IgE syndrome (HIES), is a rare primary immunodeficiency syndrome characterized by the clinical triad of high serum levels of IgE, recurring staphylococcal skin abscesses, and pneumonia with pneumatocele formation [1]. Almost all patients with HIES suffer from recurrent staphylococcal infections, beginning early in infancy and predominantly involving the skin and lungs [2]. In addition, eczematous skin lesions and dermatitis can be a dominant and early presenting feature. Both autosomal dominant and autosomal recessive forms of the disease have been identified. Mutation in the signal transducer and activator of transcription 3 (*STAT3*) gene has been shown to account for majority of the autosomal dominant and sporadic forms of HIES [2–4] whereas tyrosinase kinase 2 (*TYK2*) [5] and dedicator of cytokinesis 8 (*DOCK8*) [6] genes are implicated in the recessive forms of the disease.

Patients with autosomal dominant HIES have characteristic features based on which the National Institutes of Health (NIH) scoring has been devised [7]. Patients with higher NIH score are more likely to harbour a mutation in *STAT3* and hence can be subjected to mutation analysis [4, 8]. Alternative scoring system like the Woellner's score has been used successfully to predict *STAT3* mutation [9]. Patients with *STAT3* mutation have also been shown to have low T_H17 cell numbers [8, 9]. A combination of high NIH score together with a low T_H17 cell count thus can help in evaluating patients for *STAT3* mutation.

Material and Methods

Nineteen patients with clinical diagnosis of HIES with a NIH score of ≥20, were recruited from the Pediatric Immunodeficiency Clinic, Division of Allergy and

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Immunology, Advanced Pediatric Centre at the Postgraduate Institute of Medical Education and Research, Chandigarh after informed consent/assent. There were 18 males and 1 female (age range from 3 to 40 y, median age 12 y) from 18 unrelated families. Cases were selected on the basis of increased serum IgE, eczema, staphylococcal infections and no other well defined primary immunodeficiency (PID) associated with raised serum IgE and eczema *viz.* Ommen Syndrome, Wiskott Aldrich syndrome, immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, and Comel-Netherton syndrome. The patients were sub-grouped based on the presence or absence of *STAT3* pathogenic variants — M + ve (*STAT3* pathogenic variant present, $n = 5$) and M –ve (No identifiable *STAT3* pathogenic variant, $n = 14$). Twenty healthy controls (HC) were recruited. Three ml of heparinised blood was collected from the patients and controls by venepuncture for peripheral blood mononuclear cells (PBMCs) isolation, 1 ml blood in EDTA for DNA extraction and 1ml clotted blood for serum IgE estimation.

Genomic DNA was isolated from whole blood by Axygen blood genomic DNA miniprep kit (Axygen Biosciences, Union City, CA). Full length *STAT3* gene was amplified and sequenced using Sanger sequencing. Sequencing was outsourced to Chromus Biotech, India Pvt. Ltd., Bangalore and sequencing of amplified products was performed using 3730XL-DNA analyzer machine, BDT version 3.1 (Applied Biosciences, Foster City, CA).

PBMCs were isolated from 3ml heparinised blood using Ficoll-Hypaque Plus (Sigma Aldrich, USA) by density gradient centrifugation method. One million PBMCs were stimulated for 6 h with 10 ng/ml phorbol-12-myristate 13- acetate (PMA, Sigma Aldrich, USA) and 1µg/ml ionomycin (Sigma Aldrich) in the presence of 10µg/ml Brefeldin (Sigma Aldrich). After cell surface staining with phycoerythrin (PE) conjugated anti-CD4 antibody (BD Biosciences), cells were fixed, permeabilized and stained with Alexa Fluor conjugated anti-Interleukin17A (anti-IL17A, BD Biosciences) and FITC labelled anti-Interferon-γ (anti- IFNγ, BD Biosciences), the latter as a control for cellular activation and intracellular staining. Flowcytometry studies were performed on BD FACSCalibur using FACS Diva software. T_H17 cells were reported as percentage of total CD4+ T cells.

Descriptive methods of statistical analysis were used using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, version 15.0 for Windows). All quantitative variables were estimated using measures of central location *i.e.*, mean, median and measures of dispersion *i.e.*, standard deviation. For normally distributed data, means of groups were compared using one way ANOVA and significance between two groups was determined using post hoc Bonferroni correction. For skewed data, Kruskal-Wallis analysis was done and significance between two groups was determined using Dunn-Sidak correction.

Results

Mutations in the *STAT3* gene were found in the DNA binding domain in 3, trans-activation domain in 1 and SH2 domain in 1 patient. Four of these patients have previously been reported [10]. The fifth case is a patient with SH2 domain mutation (g.66092 T > C; c.1979 T > C; p. M660T). *TYK2* and *DOCK8* genes were not sequenced in this study.

The clinical features along with the serum IgE levels and Absolute eosinophil count (AEC) are summarized in Table 1. All five patients with *STAT3* pathogenic variants had eczema, raised IgE levels and high eosinophil counts. Pneumonia and skin abscess were documented in four and three patients respectively. Skeletal abnormalities with characteristic facies were observed in all except one patient. Only three patients had features of increased nasal width. Retained primary teeth and fracture after minimal trauma were observed only in one patient. Features like hyperextensible joints and scoliosis were not observed. Though bronchiectasis was seen in one case with *STAT3* pathogenic variant (M + ve), pneumatocele formation was not observed. Of the 5 cases with *STAT3* pathogenic variants, 2 had additional mycobacterial infection, one with a subcutaneous mycobacterial abscess in the arm, and the other had a BCG site abscess along with prominent mucocutaneous candidiasis with destruction of the tongue. The case with SH2 domain mutation presented with deep seated abscesses in the psoas and sterno-clavicular regions. Pedigree charts of the M + ve cases are shown in Fig. 1.

In M + ve group, 4 patients had NIH score > 40, while one case had NIH score < 40 and was detected because of a positive family history. Two patients with no identifiable *STAT3* pathogenic variants (M –ve group) had NIH score > 40. According to Woellner's scoring, three patients in M + ve group had score > 30 and 2 had score < 30. Only one patient had score > 30 in M –ve group. NIH score had sensitivity of 80 % and specificity of 87.5 % to detect cases with *STAT3* pathogenic variants, compared with Woellners scoring which had sensitivity of 60% and specificity of 93.75% in the present study. Of the cases in M-ve group with high NIH score, one patient had a NIH score of 44 with typical dysmorphic facies, serum IgE of 36, 800 IU/ml and history of recurrent bone fractures. The other had a score of 40 with skin abscesses, raised IgE and scoliosis.

Mean AEC in healthy controls was 218.9/mm³. AEC in HC vs. HIES patient groups were statistically significant ($p < 0.05$). However, within HIES groups, no significant difference was seen.

Serum IgE levels in HC was 632 IU/ml. The difference in serum IgE levels were statistically significant between HC and HIES patient groups.

The threshold value of normal T_H17 cell numbers expressed as a percentage of CD4+ IL-17+ T cells was determined in healthy adult control subjects (HC) and set as greater than 0.6%, with a range of 0.6–2.84 (mean 1.58 ± 0.72 and

Table 1 Distribution of various clinical parameters in patients of HIES and healthy controls

Clinical feature	HIES				HC N = 20
	NIH Score		Mutation Status		
	Group 1 (≥ 40) N = 6	Group 2 (< 40) N = 13	Group 3 (+) N = 5	Group 4 (-) N = 14	
Raised IgE*	5	8	5	10	0
Raised AEC**	5	6	4	7	2
Skin abscess	5	10	3	12	0
Pneumonia	6	7	4	10	0
Bronchiectasis	1	1	1	2	0
Coarse facies	6	3	4	5	0
Scoliosis	1	0	0	1	0
Fractures	2	0	1	1	0
Eczema	5	7	5	8	0
URI	2	9	2	9	14
Other Infections (Oral candidiasis)	3	1	4	3	0
Increased nasal width	4	3	3	4	0
NIH > 40	6	-	4	2	-
STAT3 mutation	4	1	5	0	ND

*Serum IgE levels increased 10 times above the mean of age-matched control subjects. **AEC Absolute eosinophil count >500, ND Not done; URI Upper respiratory infections; HIES Hyper IgE syndrome; NIH National Institutes of Health

median 1.63). In M –ve group, T_H17 cells were comparable or slightly lower than those seen in HC. In contrast, T_H17 cells were markedly reduced in M + ve group ($p < 0.05$) as shown in Fig. 2. Figure 3 shows the flowcytometry plots of T_H17 cells in patients with *STAT3* pathogenic variants. The difference in T_H17 cell numbers between HC and M + ve group was significant. When T_H17 cell numbers were combined with NIH scoring, the sensitivity to detect cases with *STAT3* pathogenic variants was 100% and specificity was 85.7%.

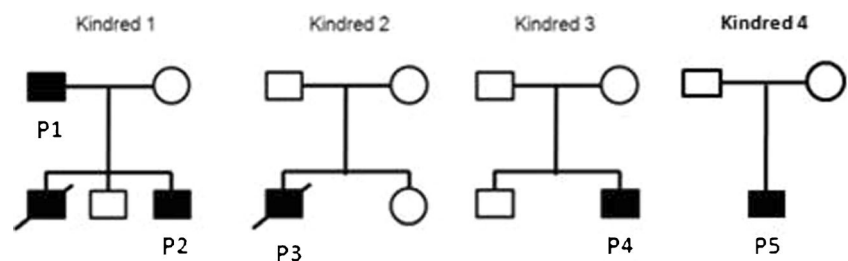
Discussion

The clinical spectrum of HIES comprises of many patients where the disease is suspected but falls short with the NIH criteria. NIH scoring is designed only for autosomal dominant form of the disease which is *STAT3* related, and the atypical/ borderline cases largely remain undefined. This study hence tried to include borderline cases with NIH score of ≥ 20 to 39

in addition to those with high scores (≥ 40). These cases were then grouped depending on the presence or absence of *STAT3* pathogenic variants and studied further. With the recent description of intermediate phenotypes of HIES arising due to genetic mosaicism within different tissues of the same individual resulting in low scores, despite having low T_H17 cells, and mucocutaneous candidiasis [11], it becomes even more relevant to study cases with low NIH scores. No genotype-phenotype correlation was observed in the index study, and similar lack of correlation between the nature of mutation and disease phenotype has been observed in previous studies [4].

In a previous study, 96 % of cases with NIH score > 40 were found to be *STAT3* mutation positive and 10 % cases were mutation negative [12]. However, in the index study, four cases with NIH score > 40 were found to have *STAT3* pathogenic variants and two had no identifiable *STAT3* pathogenic variant. Also, one patient with *STAT3* mutation had NIH score < 40. This child with low NIH score had eczema and recurrent upper respiratory infections. However, he had not

Fig. 1 Family trees of the cases with *STAT3* pathogenic variants. Patient 2 was the third child of patient 1 (His first child had expired early in life before any investigation could be done). Patients 3, 4 and 5 were the only child diagnosed in their family with *STAT3* pathogenic variants



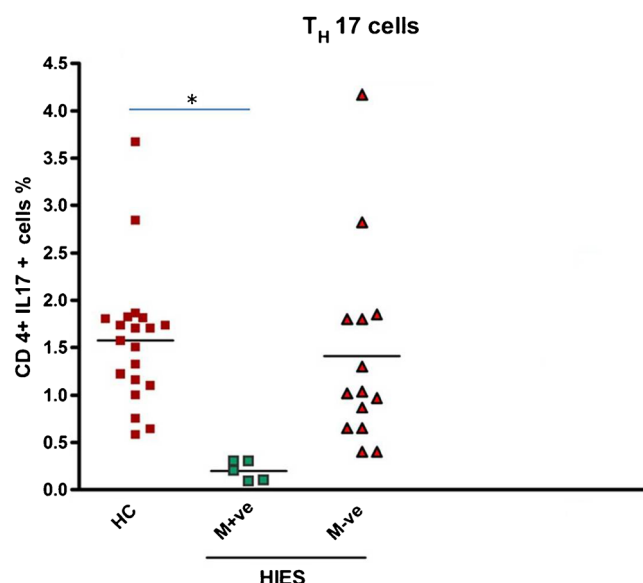


Fig. 2 Dot plots to compare CD4+ IL 17+ cells in patients and control groups. The T_H17 cell numbers in cases with *STAT3* mutation (M + ve) were markedly reduced. The difference in T_H17 cell numbers between HC and M + ve was significant ($P < 0.001$)

developed the typical skeletal features or severe infectious complications characteristic of HIES and was screened because of a positive family history. It is well known that clinical features in HIES accumulate over time as affected children get older. Also, adequate treatment with antibiotics, especially with a positive family history can forestall infectious complications, and hence a lower NIH score. NIH score had a better sensitivity compared to Woellner's score (80 % vs. 60 %) but was less specific (87.5 % vs. 93.75%) to detect cases with *STAT3* pathogenic variants. However, the number of cases in index study was small, and it is difficult to conclude on the utility of Woellner's scoring over NIH scoring or *vice versa*.

STAT3 mutations in cases with HIES affect the development of interleukin 17 (IL-17) producing T_H17 effector T cells, which are thought to play a prominent role in controlling common infectious agents observed in HIES, such as extracellular bacteria and fungi. In the index study, T_H17 cells were markedly reduced in patients with *STAT3* pathogenic variants compared to healthy controls and cases with wild type *STAT3*. However, the difference in T_H17 cell numbers between healthy controls and *STAT3* mutation negative cases was not significant. This shows that cases with *STAT3* pathogenic variants could be differentiated from healthy controls as well as from *STAT3* mutation negative cases based on T_H17 cell numbers. The sensitivity of combined NIH scores and T_H17 cell numbers was higher (100%) compared to NIH score alone (80%). However, there was no significant difference in specificity.

In a previous study by Schimke et al., T_H17 cell numbers (normal $>0.2\%$) in patients with wild-type *STAT3* were comparable with those seen in healthy control subjects [12]. In contrast, T_H17 cells were either absent or markedly reduced in patients with *STAT3* mutations. The difference in the number of T_H17 cells between patients with *STAT3* mutations and patients with wild-type *STAT3* or healthy control subjects was highly significant ($p < 0.001$). It has been reported that memory B cell numbers are also decreased in HIES [13, 14] and this finding, found in 94.5% of cases [14], has been advocated to be included in the diagnostic criteria of HIES. Memory B cells that were analyzed in one M + ve patient were found to be low (results not shown). There was no significant difference in IFN- γ producing CD4 + T cells between M + ve and M-ve group. A low T_H17 cell count is, however, a more consistent finding in *STAT3* related HIES and hence, should be included in the diagnostic criteria.

Severe Atopic dermatitis (AD) presents with eczema and increased serum IgE levels, features which are consistently

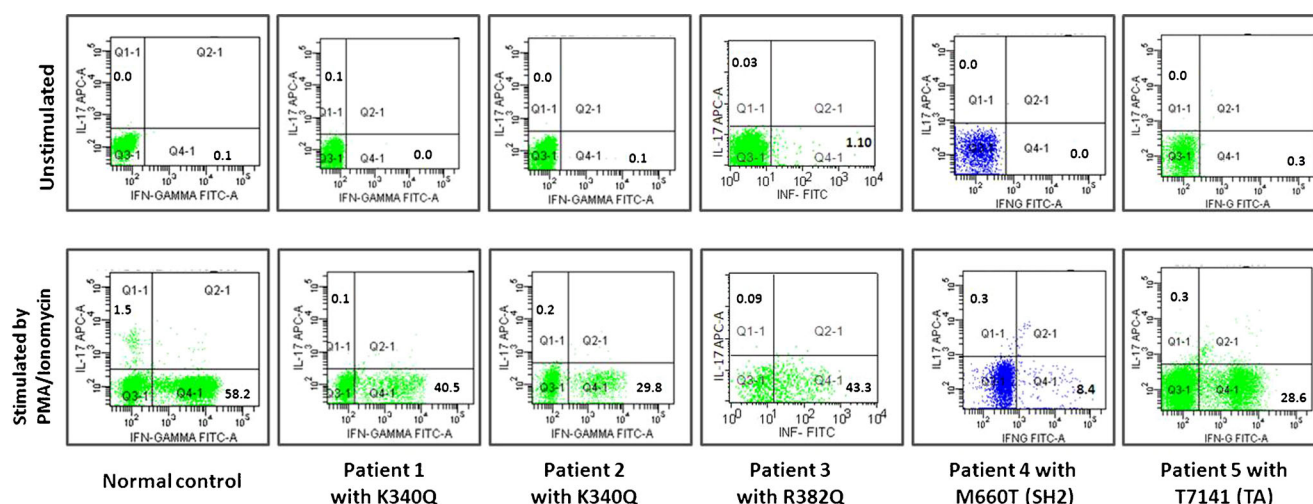


Fig. 3 Flowcytometry plots for T_H17 cells in patients with *STAT3* mutation and healthy controls. The T_H17 cell numbers were markedly reduced in patients with *STAT3* mutation compared to normal T_H17 cell

numbers in controls. The T_H17 cell numbers in five patients with *STAT3* pathogenic variants (M + ve) were 0.1, 0.2, 0.09, 0.3 and 0.3% respectively

seen in HIES and hence often comes in the differential diagnosis of a patient presenting in infancy with predominant dermatitic illness. Patients with AD however tend to have increased T_H17 cell counts in their peripheral blood as compared to cases with HIES [15].

Cases with no identifiable *STAT3* variants could represent autosomal recessive forms of HIES with *TYK2* and *DOCK8* mutations. Cases with *TYK2* mutations are characterized by recurrent cutaneous viral infections with normal number of lymphocytes but no pneumatocele formation or connective tissue and skeletal abnormalities [14, 16]. *DOCK8* immunodeficiency is characterized by lymphopenia, cutaneous viral infections like *Molluscum contagiosum* and cancers related to cutaneous viral infections [16]. In the index study, cases with absence of *STAT3* pathogenic variants did not have any history of consanguinity, recurrent viral infections or lymphopenia. There were no hepatic or neurological manifestations. Hence, clinically it is not possible to classify them further.

Conclusions

NIH scoring alone is not diagnostic of *STAT3* related HIES as the clinical features are non-specific. In the index study, patients with high NIH scores were found to be negative for *STAT3* pathogenic variants and *vice versa*. However, low T_H17 cell numbers together with NIH scores can better predict the presence of *STAT3* mutations and hence should be included in the diagnostic criteria. Children with positive family history, however, should be screened for T_H17 cell numbers even with low NIH scores. Woellners scoring was found to be less sensitive but more specific than NIH scoring. However, the sample size in the study was small and based on patients from a single centre and warrants study on a larger sample size.

Contributions BS: Helped in designing and executing the study, revised and approved the manuscript; SS: Executed the study, reviewed the literature and drafted the manuscript; SG: Helped in executing the study; RWM, SC: Guided for the execution of study; AR, DS, SurS: Provided clinical data. The final manuscript was approved by all the authors.

Compliance with Ethical Standards

Conflict of Interest None.

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References

1. Grimbacher B, Holland SM, Puck JM. Hyper-IgE syndromes. *Immunol Rev*. 2005;203:244–50.
2. Buckley RH. The hyper-IgE syndrome. *Clin Rev Allergy Immunol*. 2001;20:139–54.
3. Minegishi Y, Saito M, Tsuchiya S, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature*. 2007;448:1058–62.
4. Holland SM, DeLeo FR, Elloumi HZ, et al. *STAT3* mutations in the hyper-IgE syndrome. *N Engl J Med*. 2007;357:1608–19.
5. Minegishi Y, Saito M, Morio T, et al. Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity. *Immunity*. 2006;25:745–55.
6. Engelhardt KR, McGhee S, Winkler S, et al. Large deletions and point mutations involving the dedicator of cytokinesis 8 (*DOCK8*) in the autosomal-recessive form of hyper-IgE syndrome. *J Allergy Clin Immunol*. 2009;124:1289–302.
7. Grimbacher B, Schaffer AA, Holland SM, et al. Genetic linkage of hyper-IgE syndrome to chromosome 4. *Am J Hum Genet*. 1999;65:735–44.
8. Renner ED, Rylaarsdam S, Anover-Sombke S, et al. Novel signal transducer and activator of transcription 3 (*STAT3*) mutations, reduced T(H)17 cell numbers, and variably defective *STAT3* phosphorylation in hyper-IgE syndrome. *J Allergy Clin Immunol*. 2008;122:181–7.
9. Woellner C, Gertz EM, Schaffer AA, et al. Mutations in *STAT3* and diagnostic guidelines for hyper-IgE syndrome. *J Allergy Clin Immunol*. 2010;125:424–32.
10. Saikia B, Suri D, Goel S, et al. Hyper-IgE syndrome with a novel *STAT3* mutation- a single center study from India. *Asian Pac J Allergy Immunol*. 2014;32:321–7.
11. Hsu AP, Sowerwine KJ, Lawrence MG, et al. Intermediate phenotypes in patients with autosomal dominant hyper-IgE syndrome caused by somatic mosaicism. *J Allergy Clin Immunol*. 2013;131:1586–93.
12. Schimke LF, Sawalle-Belohradsky J, Roesler J, et al. Diagnostic approach to the hyper-IgE syndromes: immunologic and clinical key findings to differentiate hyper-IgE syndromes from atopic dermatitis. *J Allergy Clin Immunol*. 2010;126:611–7.
13. Speckmann C, Enders A, Woellner C, et al. Reduced memory B cells in patients with hyper IgE syndrome. *Clin Immunol*. 2008;129:448–54.
14. Renner ED, Puck JM, Holland SM, et al. Autosomal recessive hyperimmunoglobulin E syndrome: a distinct disease entity. *J Pediatr*. 2004;144:93–9.
15. Koga C, Kabashima K, Shiraishi N, Kobayashi M, Tokura Y. Possible pathogenic role of Th17 cells for atopic dermatitis. *J Invest Dermatol*. 2008;128:2625–30.
16. Zhang Q, Davis JC, Lamborn IT, et al. Combined immunodeficiency associated with *DOCK8* mutations. *N Engl J Med*. 2009;361:2046–55.