The Spectrum of Hypereosinophilia and Associated Clonal Disorders – A Real-World Data Based on Combined Retrospective and Prospective Analysis from a Tropical Setting

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Abstract. Objective. To determine the frequency, etiological spectrum and treatment outcome of hypereosinophilia (HE) and hypereosinophilic syndromes (HES) in a tropical setting. Methods. A retrospective analysis of hospital data of five years (January 2009 to December 2013) and a comprehensive prospective evaluation of patients presenting with HE/HES over a period of 33 months (January 2014 to September 2016) was performed. Results. HE/HES was diagnosed in a total of 125 patients during the study period with an estimated prevalence of 0.5-1 case per 100,000 population in our hospital settings. 41 patients were excluded from the final analysis due to lack of sufficient data. Infections, especially helminths were the commonest cause (34%) followed by primary/clonal HE/HES (24%) and reactive HE/HES secondary to various clonal disorders (14.3%). A lymphocytic variant of HES and FIP1L1–PDGFRA positive HES were diagnosed in 3.6% each. Imatinib-responsive BCR-ABL1 negative HE/HES constitute 7.1% in our patients. None of the clinical or routine laboratory features including the age of patients, duration of HE, presence or absence of organomegaly, hemoglobin levels, eosinophil %, absolute eosinophil count, total leukocyte count, platelet counts, serum IgE levels or presence of myelofibrosis could predict or exclude malignancy in patients with HE/HES. The absence of blasts in peripheral blood or the absence of >5% blasts in bone marrow does not exclude primary/clonal HES. Conclusions. An underlying malignancy (Primary HE/HES and neoplasms leading to reactive HES; 35.7%) is diagnosed with nearly equal frequency compared to infections (34.5%) in tropical settings. There are no hematological or serological parameters, which can reliably be used to exclude an underlying malignancy, necessitating a thorough follow-up and comprehensive work-up in patients with HE/HES.

Keywords: Hypereosinophilia; Hypereosinophilic syndromes; FIP1L1–PDGFRA; clonal hypereosinophilia; Imatinib responsive hypereosinophilia; lymphocytic variant of hypereosinophilia.

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Introduction. Hypereosinophilia (HE) defined as $>1.5 \times 10^9/L$ absolute eosinophil count (AEC) in peripheral blood and hypereosinophilic syndrome (HES) defined as HE with organ dysfunction are conditions associated with a wide spectrum of etiological factors including infections, allergic and immunological disorders, drugs and malignancies. Since its early descriptions, there were significant advances in the laboratory techniques resulting in the identification of etiological factors in a large number of cases of HES, otherwise categorized under idiopathic category. There has been significant progress in the classification systems as well. While World Health Organization (WHO) system deals purely with clonal causes, the definitions and classification proposed by International Cooperative Working Group on Eosinophil Disorders (ICOG-EO) appear to be a comprehensive system dealing with clonal and non-clonal disorders. The introduction of tyrosine kinase inhibitors and other newer drugs in the treatment of HES is yet another breakthrough in this field. Despite all progress, the diagnosis and treatment of HES appear complicated due to various reasons. These include a large number of secondary causes; the wide spectrum of molecular abnormalities; co-occurrence of non-clonal and clonal causes and the absence of any definite morphological or immunophenotypic features differentiating clonal from non-clonal conditions. The presence of numerous infective agents and the lack of availability of laboratories performing a comprehensive workup of HES make it further difficult in tropical countries. There is a scarcity of published data on the HES from these regions. Knowledge of the spectrum of etiological factors is an absolute necessity for generating a consensus opinion on the essential investigations required, and for developing a management protocol suitable for the socio-economic conditions prevalent in this part of the world.

The aim of the study was to perform a comprehensive clinico-pathological evaluation of cases of HE/HES over a period of nearly eight years in an attempt to determine the relative frequency and treatment outcome with a focus on clonal/primary hypereosinophilia and secondary/reactive hypereosinophilia associated clonal disorders.

Materials and Methods. The study was conducted in the Department of Hematology in association with the departments of adult clinical hematology and pediatric hematology/oncology units from January 2009 to September 2016 (Total 93 months). The operational definition of hypereosinophilia used to recruit patients in our study was $>1.5 \times 10^9/L$ absolute eosinophil count (AEC) (and eosinophils $>5\%$) in peripheral blood. Eosinophil precursors were also included for calculating AEC. The eosinophil $\%$ was determined by manually counting a minimum of 200 leukocytes in the peripheral smear. The duration of HE, presence or absence of clinical features related to organ involvement was not considered for initial enrolment. The patients with tissue HE in the absence of peripheral blood HE were excluded from the study.

The retrospective analysis was performed using hospital records of five years (January 2009 to December 2013). The clinical features, laboratory findings, treatment and follow up details were retrieved from the clinical record files for retrospective analysis. A comprehensive prospective evaluation of patients presenting with HE/HES was performed over a period of 33 months (January 2014 to September 2016).

Patients were evaluated with detailed history, clinical examination, complete haemogram, serological tests (anti-nuclear antibody/ANA, anti-neutrophil cytoplasmic antibody/ANCA, IgE levels, parasite serology), skin hypersensitivity test for aspergillus, stool examination, morphological evaluation of peripheral blood and bone marrow, flow cytometry, reverse transcriptase PCR for $TCF3-PBX1$, $ETV6-RUNX1$, $KMT2A-AFF1$, $RUNX1-RUNX1T1$, $CBFB-MYH11$, $BCR-ABL1$, $FIP1L1-PDGFR$, $ETV6-PDGFR$ translocations. Amplification-refractory mutation system (ARMS) PCR for $JAK2 V617F$ mutation and fluorescent in situ hybridization (FISH) for $PDGFR$, $PDGFRB$ and $FGFR1$ gene rearrangements were also performed. The investigations were decided based on clinical findings, preliminary investigations result as well as response to therapy. Serological tests for parasites include IgM and IgG antibodies for Trichinella, Toxoplasma, Toxocara, Echinococcus and antigen detection for Microfilaria. These tests detect both active and past infections, and the
interpretation depends on the titer and type of antibody positivity. Patients were investigated in a stepwise manner to exclude reactive or secondary causes of eosinophilia followed by evaluation for clonal conditions.10,11

Six-color flow cytometry (antibodies from BD Biosciences, BD Canto II flow cytometer and BD FACS Diva software, San Jose, CA) was used for the evaluation of acute leukemia and lymphoproliferative disorders. Peripheral blood was also used to evaluate the presence of T cell subsets with abnormal immunophenotype. T cell immunophenotype was simultaneously studied in voluntary healthy control samples (n=25) with each batch of patients. A minimum of 1 x 10^5 T cells was acquired, gated for cytoplasmic CD3 and/or surface CD7 positive cells; and analyzed for the presence of abnormal T cells. FISH was performed using Vysis 4q12 tricolor rearrangement probe (Abbott molecular, Illinois, USA), Vysis PDGFRB break - apart probe (Abbott molecular, Illinois, USA) and Poseidon™ Repeat Free™ FGFR1 (8p12) break-apart probe (Kreatech biotechnology, Amsterdam, Netherlands) respectively. A final categorization was attempted in each case considering the clinical scenario, investigation results, follow up and response to treatment.

The final categorization was based on consensus classification by ICOG-EO. According to this classification, HE (Peripheral blood absolute eosinophil count >1.5x10^9/L without end-organ damage) is classified into hereditary/familial HE, primary/clonal/neoplastic HE (eosinophils are clonal), reactive/secondary HE (eosinophils are non-clonal or reactive) and HE of undetermined significance. HES (HE as defined above with features of end-organ damage attributable to HE) is classified into idiopathic HES (no definite cause identified), primary/neoplastic HES and secondary/reactive HES. In addition, the classification incorporates two other categories (specific syndromes and several single-organ restricted conditions associated with HE). Primary/neoplastic HE and HES incorporates all the entities described in the WHO classification of hematopoietic neoplasms.4

Discrete categorical data are presented as n (%); continuous data given as median, range and interquartile range (IQR). The comparison of two groups with skewed data was compared using Mann Whitney test, and those with normally distributed data were compared using student t-test. The comparison of categorical data between two groups was performed by Chi-square test. All statistical tests are two-sided and performed at a significance level of <0.05. Statistical analysis was performed using SPSS for Windows (version 22.0; SPSS Inc., Chicago, IL, USA). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Results. A diagnosis of HE/HES was made in a total of 125 patients over a period of 93 months between January 2009 and September 2016. Among these, 41 patients were excluded from final analysis due to lack of necessary work-up required for the final categorization. Excluded cases include 11 patients of chronic myeloid leukemia (CML) on imatinib. Out of the remaining 84 cases, 55 (65.5%) patients were enrolled prospectively over a period of 33 months and in the remaining patients, data were collected retrospectively. The demographic details and the hematological findings of patients included in the final analysis are summarized in Table 1.

The spectrum of HE/HES (n=84). A final subcategorization into HE, HES and specific syndromes associated with HE as proposed by ICOG-EO classification system4 was attempted after considering the clinical data, laboratory findings, and the treatment outcome. Majority of the patients (n=53; 63%) did not have eosinophilia associated organ dysfunction (HE). Twenty-seven patients (32%) were classified as HES, and another 5% had specific syndromes/single organ disorder with HE [Eosinophilic granulomatosis with polyangitis/EGPA (n=2), Job syndrome (n=1), Cutaneous eosinophilic vasculitis (n=1)]. Fourteen patients had moderate eosinophilia defined as 1.5-5.0x10^9/L.12 Among these, ten patients (71.4%) had reactive HE/HES while rest of the patients had clonal HE/HES. Rest of the patients (n=70; 83.3%) had severe eosinophilia (>5x10^9/L). The etiologic spectrum associated with HE/HES is summarized in Figure 1 and Table 2.

Fever was the most common symptom noted in 42% (n=35) of the patients. The organ systems
affected and the clinical features are summarized in Table 3. Children and adolescents (≤ 18 years) in Table 1. Demographic details and hematological parameters of patients (included in final analysis) with HE/HES.

| Table 1. Demographic details and hematological parameters of patients (included in final analysis) with HE/HES. |
| No. of patients | 84 |
| No. of children and adolescent with HE (≤18 years) | 25 (29.8%) |
| Male: Female ratio | 1:8:1 |
| Median (IQR) | Range |
| Age (Years) | 33 (15-48) | 1-71 |
| Duration of HE (months) at presentation | 2 (1-6) | 0.25-48 |
| Hematologic parameters | |
| Hemoglobin (g/L) | 111 (94.8 – 131) | 34 – 182 |
| Total leukocyte count (x10⁹/L) | 33.9 (19.6 – 51.6) | 6.6 – 407.3 |
| Platelet count (x 10⁹/L) | 250 (179.2 – 355.7) | 12 – 614 |
| Peak Eosinophil (%) documented | 53 (28-75) | 8 – 92 |
| Peak AEC (x10⁹/L) documented | 16.2 (7.7 – 29.8) | 1.9-135 |
| Bone marrow blast (%) | 2 (1-3) | 0-84 |
| Bone marrow eosinophil (%) | 25 (14-48) | 4-79 |
| Number of patients with anemia (%) | 52 (62%) |
| Number of patients with thrombocytopenia (%) | 15 (18%) |
| Number of patients with thrombocytosis (%) | 11 (13%) |
| Number of patients with lymphocytosis (>4x10⁹/L) (%) | 26 (31%) |
| Number of patients with blasts in the peripheral blood (%) | 11 (13%) |
| Number of patients with bone marrow fibrosis (%) | 9 (10.7%) |

* This data was available only in 70 patients where bone marrow examination was performed. IQR-inter quartile range, AEC – absolute eosinophil count.

Figure 1: Spectrum of causes of hypereosinophilia/hypereosinophilic syndrome (EGPA - Eosinophilic granulomatosis with polyangiitis; DRESS - drug reaction with eosinophilia and systemic symptoms; L-HES – lymphocytic variant of HES; HE/HES-N – Neoplastic HE/HES; HE-US – HE of undetermined significance; I-HES – idiopathic HES).
Table 2. Causes of HE/HES (n=84).

| Category                        | HE (n=53) | HES (n=27) |
|---------------------------------|-----------|------------|
| **I. Reactive causes (n=52)**   |           |            |
| Infection (n=29)                |           |            |
| Microfilaria (Positive for antigen) | 3         | -          |
| Trichinella (High antibody titre) | 3         | -          |
| Giardiasis (Positive for cyst in stool) | 3         | -          |
| Toxocara (High antibody titre)  | 1         | -          |
| Hydatid                         | 1         | -          |
| Histoplasma (Organisms in marrow and lymphnode) | 1         | -          |
| Possibly helminthic (Response to anti-helminthic drugs) | 13        | 4          |
| **Neoplasm associated reactive HE (n=9)** |           |            |
| T-NHL (includes PTCL,AITL,CTCL,T NHL-Nos) | 3         | 2          |
| T-LGL**                         | 1         | -          |
| T-ALL                           | 1         | -          |
| Hodgkin-Lymphoma                | 1         | -          |
| Angiolympoid hyperplasia with eosinophilia | -         | 1          |
| **Autoimmune (n=4)**            |           |            |
| Rheumatoid arthritis,           | 1         | -          |
| Autoimmune hepatitis            | 1         | -          |
| Autoimmune hemolytic anemia     | 1         | -          |
| Vasculitis                      | 1         | -          |
| **Evidence of allergy and helminthic infection** | 3         | -          |
| Allergy/asthma                  | 3         | -          |
| Lymphocytic variant (L-HES)     | 3         | -          |
| **DRESS syndrome**              | 1         | -          |
| **II. Neoplastic causes (n=20)**|           |            |
| AML with HE*                    | 9         | -          |
| Philadelphia positive (BCR/ABL1) chronic myeloid leukemia | 3         | -          |
| PDGFRA rearranged neoplasms (+ve) | 1         | 2          |
| JAK2 V617F positive myeloproliferative neoplasms | 1         | -          |
| JMML with HE                    | 1         | -          |
| Unclassified (imatinib responsive – BCR-ABL1 negative) | 1         | -          |
| **III. Idiopathic/ Unclassified (n=8)** |           |            |
| **IV. Syndromes associated with HE/HES (n=4)** – EGPA (n=2), Job syndrome (n=1), Cutaneous eosinophilic vasculitis (n=1). |           |            |

T-LGL - T cell large granular lymphocyte leukemia, T-ALL – T- cell acute lymphoblastic leukemia, PTCL – Peripheral T cell lymphoma, AITL – angioimmunoblastic T cell lymphoma, CTCL – cutaneous T cell lymphoma, T NHL- T- non-Hodgkin lymphoma **the patient with T-LGL also had evidence of autoimmunity due to presence of antinuclear antibody positivity (nucleolar pattern) by indirect immunofluorescence and Scl-70 antibody positivity. #t(8;21) positive in 1 & inv(16) in 2 patients. $Includes a patient with medullary carcinoma of thyroid and a patient with mixed phenotype acute leukemia where cytogenetic testing was not available.

Table 3. Organ systems affected in patients with HES and HE associated specific syndromes* (n=31).

| Organ system       | Features                                                                 |
|--------------------|---------------------------------------------------------------------------|
| Skin               | 58% (n=18) Eczema, angioedema, rash, erythema, vesicles, leukocytoclastic vasculitis, skin nodules. |
| Respiratory        | 54.8% (n=17) Dyspnea, wheeze, cough, pleural effusion, pulmonary thromboembolism, restriction in pulmonary function tests, pulmonary infiltrates on imaging |
| Constitutional     | 35.5% (n=11) Fever                                                        |
| Spleen             | 29% (n=9) Splenomegaly                                                   |
| Hepatic            | 25.8% (n=8) Hepatomegaly and hepatitis                                     |
| Cardiac            | 25.8% (n=8) Cardiac failure, pericardial effusion, mitral regurgitation, myocardial infarct |
| Gastrointestinal   | 22.6% (n=7) Diarrhea, vomiting, abdominal pain                             |
| Hematologic        | 12.9% (n=4) Bone marrow fibrosis, autoimmune hemolytic anemia             |
| Vascular           | 9.7% (n=3) Deep vein thrombosis of popliteal vein, thrombosis of tibioperoneal artery, pulmonary artery thrombosis |
| Rheumatologic      | 6.5% (n=2) Arthralgia                                                     |

* Includes symptoms and signs considered for diagnosing HES as well as associated clinical manifestations.
constituted nearly 1/3rd of the study population. In this group, infections (12/25; 48%) were the commonest causes, followed by neoplasms (6/25; 24%). Autoimmune (n=2) disorders, allergy (n=2), Job syndrome (n=1), drug reaction with eosinophilia and systemic symptoms due to phenobarbital (DRESS) (n=1) and EGPA (n=1) were other causes of HE/HES in this age group.

Secondary HE/HES associated clonal disorders (n=12). HE was diagnosed as a reactive phenomenon secondary to various benign/malignant neoplasms in 10.7% (n=9) of patients (Table 2). A lymphocytic variant of HE (L-HES) was diagnosed in another three patients (3.6%), which constituted 11% of cases with HES. They were diagnosed following demonstration of clones of mature T cells with abnormal immunophenotype [CD3+CD4+CD5+ T cells (n=1) and CD4+CD8+T cells (n=2)], a dramatic response of eosinophilia to steroids and the requirement of long-term low dose steroids for controlling HE. However, T cell receptor clonality studies were not performed.

Primary or Clonal or Neoplastic HE/HES (n=20). A “clonal” HES (n=20; 23.8%) was diagnosed in the presence of either a cytogenetic abnormality or bone marrow morphological evidence of a myeloid neoplasm. Various causes are summarized in Table 2. Overall imatinib responsive HE/HES (BCR-ABL1 negative) constitute 7.1% (n=6) of HE cases. Of these, FIP1L1-PDGFRA positive HE/HES was diagnosed in 3.6% (n=3) of patients (Figure 2).

Figure 2: (A) Peripheral blood smear of a patient with Imatinib responsive hypereosinophilia showing bilobed and trilobed eosinophils and myelocytes. (40x, May Grunwald Giemsa stain). (B) Bone marrow trephine biopsy showing hypercellular marrow spaces with marked increase in eosinophils and precursors (Hematoxylin and eosin, 40x). (C) Reticulin stain shows myelofibrosis (Grade 2/3; WHO). (D) Interphase FISH using tricolor FIP1L1-PDGFRA rearrangement probe showing deletion of CHIC2 gene (orange signals) in a case of FIP1L1-PDGFRA positive HES.
They were all males with age ranging from 25-40 years. The eosinophil % and AEC ranged from 54-62% and 14.3-23 x 10^9/L respectively. In one of them, HE was detected during the workup of fever, while the other two patients presented with organ dysfunction (cardiac failure and deep vein thrombosis). All of them had moderate splenomegaly. Other three patients with HES were categorized as imatinib responsive (BCR-ABL1 negative) HES as their symptoms (cardiac symptoms in two and bone marrow fibrosis in the third patient) responded very well to low doses (100mg) of this tyrosine kinase inhibitor. FIP1L1-PDGFRα was negative in one of these patients but was not tested in the other two cases due to its non-availability during that period.

**HE with malignancy (n=30; 35.7%) vs. HE without malignancy (n=54; 64.3%).** HE was associated with malignancy as a reactive or clonal process in 35.7% of patients. Malignancy was diagnosed in 50% (7/14) of patients with moderate eosinophilia, while it was diagnosed in 32.8% (23/70) with severe eosinophilia. A comparison of various parameters was performed between two groups of patients (HE with malignancy vs. HE without malignancy; **Table 4**).

Between two groups, there was no significant difference for absolute eosinophil count, age or duration of HE. HE with malignancy had significantly lower hemoglobin levels (P=0.013), eosinophil % (P=0.0004) and platelet counts (P=0.0016); higher levels of total leukocyte count (P=0.035), higher frequency of bone marrow fibrosis (P=0.02) and organomegaly (P=0.04). However, due to the significant overlap between groups, except for the presence of blasts in peripheral blood and >5% blasts in the bone marrow; none of the other clinical or routine laboratory features, including the age of patients, duration of HE, presence or absence of organomegaly, hemoglobin levels, eosinophil %, absolute eosinophil count, total leuкоcyte count, platelet counts, serum IgE levels or presence of myelofibrosis, could distinguish between patients with or without malignancy.

**Treatment and outcome of HE/HES.** The treatment modalities employed are summarized in **Figure 3**. Anti helminthic/parasitic medications including albendazole, mebendazole, diethylcarbamazine (DEC), metronidazole and tinidazole either alone or in combination; and steroids were the drugs most commonly used. Imatinib was used in seven cases of which three patients received it empirically with good response. Following treatment, patients were followed up for the symptomatic response, response in AEC and recurrence of HE. A follow up was available in 79.8% (67/84) of patients, and the duration of follow-up ranged from 15 days to 58 months (median nine months). Among these, 94% (n=63) patients showed a response to therapy with 13% (n=9) of them showing fluctuating eosinophil levels. Two patients (AML with HE and idiopathic HE) expired during the follow-up period, while another two patients (polycythemia vera with HE and Job syndrome) continued to show HE during the last follow-up. Twenty-four (35.8%) patients required long-term treatment for the control of HE. These include patients with autoimmune disorders.

**Table 4.** The demographic profile and hematological parameters of patients with and without malignancy.

| Parameter                              | HE with malignancy | HE without malignancy | p value |
|----------------------------------------|--------------------|-----------------------|---------|
| Age (years)                            | 40 (1-68)          | 30 (1-71)             | 0.251   |
| Duration of HE (months)                | 1 (0.5-45)         | 2 (0.25-48)           | 0.99    |
| Hemoglobin (g/L)                       | 102 (34-182)       | 116 (62-181)          | 0.013*  |
| Max TLC x10^9/L                        | 41.4 (13.7-407.3)  | 28.2 (6.6-151.4)      | 0.033*  |
| Eosinophil%                            | 34 (8-84)          | 65 (14-92)            | 0.0004* |
| Max AEC x10^9/L                        | 14.3 (3-135)       | 19.3 (1.9-128.7)      | 0.32    |
| Platelet x10^9/L                       | 179 (12-517)       | 286 (26-614)          | 0.00162*|
| Peripheral blood blast%                | 0 (0-84)           | 0 (0-0)               | 0.008*  |
| Bone marrow blast%                     | 4 (0-84)           | 2 (0-5)               | 0.005*  |
| Bone marrow Eo%                        | 22 (7-59)          | 29 (4-79)             | 0.06    |
| No. of patients with bone marrow fibrosis | 7                  | 2                     | 0.02*   |
| IgE levels (IU/ml)                     | 493 (5-10000)      | 2141 (50-10000)       | -nt     |
| No. of patients with organomegaly (hepatomegaly and/or splenomegaly) | 15                 | 14                    | 0.04*   |

AEC - absolute eosinophil count, TLC - total leukocyte count, Eo – eosinophil, nt – not tested due to lack of sufficient number of patients in one of the groups. *statistically significant.
Figure 3. The spectrum of therapeutic agents used in the treatment of hypereosinophilic syndromes (n=67) (DEC – diethylcarbamazine).

[includes HE associated specific syndromes like EGPA) (n=5), L-HES (n=3), idiopathic HES (n=3), allergy (n=3), CML (n=2), imatinib responsive HE (n=3), FIP1L1-PDGFRa+ve HES (n=2), cutaneous T cell lymphoma (n=1), T-cell large granular lymphocyte leukemia (n=1) and unclassified HES (n=1)]. Oral or inhalational low dose steroids (10 patients), other immunosuppressants like azathioprine, cyclophosphamide, and mycophenolate mofetil with/without steroids (n=7) and imatinib (n=7) were given singly or in combination to these patients.

Discussion. The estimation of prevalence of HE/HES is difficult due to the lack of clear consensus and changes in its definition over the years. Based on surveillance, epidemiology, and end result (SEER) database of the National Cancer Institute, the age-adjusted incidence of HES is 0.18 per 100000.13 These may not represent the true incidence in tropical countries with high prevalence of parasitic infections; and unfortunately, there is no data regarding the same from several tropical countries including India. During our study period, we encountered a total of 125 patients (over 93 months) with HE/HES. Since the hospital received an average of 200,000 patients per month (6000-7000 patients/day), this number translates to 0.5 to 1 case/100,000 hospital population. HES was diagnosed in 1/3rd of these patients. The referral bias and the partially retrospective nature of study make this figure likely to be an underestimate, and HE/HES appears to be frequently encountered in this region.

The consensus proposal from Valent et al.4 has refined the definitions of HE and HES. However, the cut off levels of HE and duration of disease is still arbitrary. Many patients with AEC below the proposed levels and duration less than one month may still require workup and treatment even in the absence of organ dysfunction. This is especially important in regions with poor socioeconomic conditions, where there is a difficulty in the follow-up of patients. The present study includes all patients with HE/HES irrespective of the duration. Overall, 63/84 (75%) of the patients had persistent HE (≥ 1 month). In three patients with HES, duration of HE was less than one month. Of these, one patient developed pulmonary thromboembolism, myocardial infarction and
expired within two weeks. Other two patients presented with thrombosis and severe eczema requiring intervention.

In our study, infections, especially helminths were the commonest cause of HE as well as HES. We had patients with malignancy (acute lymphoblastic leukemia, diffuse large B cell lymphoma) and atopy/allergy, which was complicated by parasitic infections. The diagnosis was predominantly based on the response to anti-helminthic drugs (albendazole, mebendazole, diethylcarbamazine/DEC) in others. However, a definite organism could be demonstrated in only 12/29 (41.4%) patients with infection (Table 2). It is challenging to demonstrate a definite organism in the majority of the patients, which requires a wider panel and more sensitive laboratory investigations. Moreover, many of these infections remain subclinical with HE as the only manifestation. However, in symptomatic patients, a detailed history of travel, exposure and the type of symptom complexes will help to identify the cause of HE/HES. In resource-limited settings, an empirical course of anti-helminthic therapy may safely precede detailed work-up.

HE preceded the diagnosis of T lineage acute lymphoblastic leukemia, Hodgkin Lymphoma and cutaneous T cell lymphoma in one patient each. HE can mask the underlying neoplasm and may precede, occur simultaneously or succeed in various neoplasms especially acute lymphoblastic leukemia. A thorough follow-up is must, and a bone marrow examination and a detailed evaluation should not be delayed in any patient with the slightest suspicion of an underlying neoplasm.

Three patients (4%) showed HE associated with allergy/asthma. Another three (4%) patients had evidence of both allergy and helminthic infection. They showed a dramatic response to anti-helminthic drugs; however, required low dose inhalational or oral steroids for control of allergic/asthmatic symptoms. Allergic disorders are common in this part of the world with the reported prevalence of approximately 30%. The reported prevalence of asthma is 7.5%, and skin allergy is 5.8%. The common precipitants of allergic disorders include dust, seasonal changes, and food substances. It is imperative to take a detailed history of allergy and exposure to various allergens while dealing with a case of HE/HES.

L-HES is a distinct variant of reactive HE characterized by the presence of HE in association with the secretion of IL-5 from expanded immunophenotypically aberrant clonal T cells, most commonly CD3-CD4+ T cells. The prevalence of L-HES in our study was 3.6% (3/84) among all cases with HE or 11% (3/27) among cases with HES. Since the first report of clonal proliferation of type 2 helper cells in patient with HE, there have been several case reports and small series of cases describing this entity. The reported prevalence ranges from 17-26%, Despite several reports and reviews, there is still a lack of consensus in the diagnosis of this entity. Studies have used immunophenotype abnormalities or presence of clonal T cell receptor rearrangements alone or both for diagnosing L-HES. In our study, peripheral blood flow cytometry on normal controls (n=25) and patients with various infections did not show CD3-CD4+, CD4+CD8+ and CD2- T cells; but showed variable proportion of CD4-CD8-, CD4+CD7-, CD7-, CD5- subsets of T cells as these may represent NK cells, gamma delta T cells, NK/T cells or could be part of the normal immunological response as seen in various infections. These normal alterations in the immunophenotype should be considered before a diagnosis of L-HES is made especially in the absence of clonality studies in tropical countries with high prevalence of infections. Similarly, it is also important not to over diagnose L-HES based on the isolated presence of clonal population of T cells without immunophenotypic abnormalities as they can be identified in normal population as well.

Overlap HES refers to patients with overlapping features of HES and EGPA or those with single organ disease (like gastrointestinal disease, episodic angioedema, eosinophilic fasciitis) with HE. We had three patients with overlap HES (two patients with EGPA and one patient with cutaneous eosinophilic vasculitis). In these cases, the cause-effect relationship, i.e. whether the HE causes single organ dysfunction or, HE is a manifestation of the primary disease itself remains debatable.

Among “clonal” HES, FIP1L1-PDGFRα+ve HES is the most common cause. Other PDGFRα, PDGFRβ, FGFR1 and JAK2 related translocations are reported in very few (<5) patients or in single individuals. In our study FIP1L1-PDGFRα+ HE/HES was diagnosed in 3.6% of patients.
compared to the reported frequency of 3-17% in various recent studies.\textsuperscript{29–31} JAK2 V617F associated HE-N was seen in a single patient (1.2%) compared to the reported frequency of 4% in the literature.\textsuperscript{30}

The presence of nearly 50 translocations and several mutations involving PDGFRα, PDGFRβ, FGFR1, JAK2 and several other genes associated with clonal HES; and due to the possibility of occurrence of various malignancies underlying reactive HE/HES, it is imperative to identify markers which can predict malignancy associated HES. This is especially important, as our study shows that an underlying malignancy (n=30; 35.7%) in the form of clonal HE/HES (n=20); Hodgkin or non-Hodgkin lymphomas and leukemias leading to reactive HE/HES (n=9); or other miscellaneous malignancies like carcinomas (n=1) causing paraneoplastic HE, is as frequent as various infections (n=29; 34.5%). Our study shows that the patients with malignancy had significantly lower Hb levels, eosinophil %, and platelet counts; higher levels of total leukocyte count (TLC), peripheral blood or bone marrow blast %, the incidence of bone marrow fibrosis and a higher proportion of patients with organomegaly. The median IgE levels were also higher in patients without malignancy. However, the overlap in the values between the two groups makes them less useful to predict or exclude malignancy. Again, though the presence of blasts in peripheral blood and >5% blasts in the bone marrow was exclusively seen in malignancy; their absence did not exclude clonal HE/HES. Blasts, mast cells, and fibrosis are reported to be more frequent in bone marrow biopsies of patients with FIP1L1-PDGFRα translocation.\textsuperscript{32} But, none of our patients with FIP1L1-PDGFRα translocation had blasts in the peripheral blood or bone marrow blasts >5%. Only one of them had anemia while platelet counts were normal in all of them. However, 50% of Imatinib responsive HES (BCR-ABL1 negative) patients had myelofibrosis, probably the most useful morphological indicator in our study. Elevated serum vitamin B12 and tryptase levels are other parameters suggested to be associated with myeloproliferative neoplasms\textsuperscript{33} but were not evaluated in the current study.

An exact categorization could not be possible in eight patients (HE of undetermined significance/Idiopathic HES/unclassified) due to various reasons. The inclusion of cytogenetic testing, a complete panel of FISH testing for PDGFRα, PDGFRβ, FGFR1, JAK2 rearrangements and molecular testing for clonal T cell clones might reveal a cause in many of these cases. The improvement in the knowledge about pathobiology of HE/HES and availability of advanced laboratory technology including next-generation sequencing is expected to solve the mystery behind several cases of idiopathic HES/HE of undetermined significance in future. Another limitation of our study is the compilation of retrospective with prospective data probably resulting in selection bias and underestimating the true prevalence of HE/HES.

Compared to two large studies from National Institute of Health\textsuperscript{27} and Mayo Clinic\textsuperscript{34} respectively, our study shows very high frequency of secondary/reactive HE/HES (10% and 46% vs. 62%) and neoplastic/clonal/myeloproliferative HE/HES (10% and 17% vs. 24%), very low frequency of idiopathic HE/HES (47% and 32% vs. 9.8%) and low prevalence of L-HES (14.8% and 4% vs. 3.6%).

Conclusions. HE/HES appears to be an under-reported public health problem in tropical settings with an estimated prevalence of 0.5-1-case/100,000 population in hospital settings. Infections especially helminths are the commonest cause of HE/HES in our study, and should be excluded even in patients with other causes of HE. The spectrum of infections is so wide that the demonstration of the specific infective agent is often difficult in resource-limited settings; necessitating an empirical course of anti-helminths in most of the patients. In contrary to the general perception in tropical countries, an underlying malignancy is diagnosed with nearly equal frequency compared to infections. An underlying malignancy is highly likely in patients with presence of blasts in peripheral blood, >5% blasts in bone marrow and bone marrow fibrosis. But there are no hematological or serological parameters, which can reliably be used to exclude an underlying malignancy, necessitating a thorough follow-up and comprehensive work-up in patients with HE/HES.

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