Received Date : 28-Apr-2016
Revised Date : 13-Sep-2016
Accepted Date : 24-Sep-2016
Article type : Letter to the Editor

Local chemokine profiling in eosinophilic esophagitis: the Synthetic Absorptive Matrix test

Eleanor Minshall[1] PhD, Harsita Patel[2] MBBS, Nicholas Francis[3] FRCPath, Trevor Hansel[4] FRCPath, John O. Warner[2] FRCPCH, Robert J. Boyle[2] PhD, John Fell[5] FRCPCH, Jenny Epstein[5] PhD.

[1]Department of Paediatric Allergy, Cambridge University Hospitals NHS Foundation Trust, [2]Paediatrics and the NIHR Biomedical Research Centre, [3]Histopathology, St Mary's Hospital and [4]The Imperial Clinical Respiratory Research Unit, Imperial Healthcare NHS Trust, [5]Department of Paediatric Gastroenterology, Chelsea and Westminster Hospital, London.

Author for correspondence:
Dr Jenny Epstein
Department of Paediatric Gastroenterology
Chelsea and Westminster Hospital
369 Fulham Road
London SW10 9NH
Email: j.epstein@imperial.ac.uk

Funding Source: This work was made possible by a grant from the ICHT NIHR Biomedical Research Centre. This body had no input in the study design, collection, analysis or interpretation of data, in the writing of the report or decision to submit the article for publication.

Keywords: Eosinophilic esophagitis, eotaxin-1, MCP-4, allergy, inflammation, esophagus, synthetic absorptive matrix, chemokines, cytokines

Summary

We describe a novel method of sampling the esophageal lining fluid in children and show that levels of eotaxin-1 and MCP-4 differentiate those children with a histological diagnosis of EoE from those without.

Abbreviations:

CV: Coefficient of variation

EoE: Eosinophilic esophagitis

GERD: gastro-esophageal reflux disease

Hpf: high powered field

IQR: interquartile range

MLF: mucosal lining fluid

PPI: proton pump inhibitor

SAM: synthetic absorptive matrix

Local chemokine profiling in eosinophilic esophagitis: the Synthetic Absorptive Matrix test

To the Editor:

Eosinophilic esophagitis (EoE) is a local disorder with increased expression of various chemokines and cytokines which reflect a predominant Th2-type inflammatory response. This profile of mediator expression drives the recruitment and *in situ* activation of eosinophils and is associated with increased levels of eotaxin-1, eotaxin-3, TSLP, and IL-5[1-3]. There has been a recent interest in minimally-invasive methods in the diagnosis and surveillance of EoE[4]. Previous studies have described a technique for sampling airway fluid within the respiratory tract using a synthetic absorptive matrix[5] (SAM). This device is composed of a hydroxylated polyester of high absorbency which facilitates the measurement of local cytokines and chemokines within the mucosal lining fluid. Our aim was to adapt this device for the determination of mediators within the esophagus, and to assess whether it was able to distinguish between children with active EoE and those with a similar clinical presentation but no histological evidence of EoE.

Children (n = 10) with symptoms suggestive of EoE were recruited within a tertiary gastroenterology clinic. Their clinical characteristics are documented in Supplementary Table 1. The collection and analysis of esophageal lining fluid for chemokines and cytokines using SAM and multi-array technology, and immunohistochemistry for resident cell populations are given in the Supplementary Methods section.

There was no difference in demographics, clinical characteristics or medication use between children with active EoE and controls (Supplementary Table 1; $p > 0.05$). The profile of chemokine and cytokine expression within esophageal lining fluid is shown in Supplementary Figure 1 and there was no significant difference in the detectability of chemokines and cytokines between cases and controls other than for eotaxin-1 and MCP-4 ($p<0.05$). Eotaxin-1 was significantly raised in EoE (median 39.3 pg/ml, cases n = 6; 6.4 pg/ml, controls n = 4; $p<0.02$; Figure 1) as was MCP-4 (median 36.7 pg/ml, cases n = 6; 3.8 pg/ml, controls n = 4; $p<0.05$). Median concentrations of chemokine and cytokine immunoreactivity in the esophageal lining fluid in active EoE and controls are shown in

Table 2 of the Supplementary data. In the esophagus, multiple mediators (Eotaxin-3, IL-8, IP-10, MCP-1, MDC, MIP-1β, TARC, IL-13, and TNFα) had raised median concentrations levels in cases compared to controls, but not at a statistically significant level ($p>0.05$). Mediator concentrations in saliva and duodenal samples were generally lower in comparison to nasal and esophageal mediators (Supplementary Figure 2). Certain mediators (IL-8, IL-10 and TNFα) had high detectability across all sample locations. When correlating esophageal mediator levels with nasal, saliva and duodenal levels, no relevant, significant associations were seen ($p > 0.05$). Children with active EoE had greater numbers of CD3, CD4 and CD117-positive cells, and intact and degranulated eosinophils staining with chromotrope 2R (Supplementary Table 3).

In this proof of concept study we found that cytokines and chemokines can be determined locally within the esophageal lining fluid at endoscopy using esophageal SAM. These levels did not correlate with those found in either saliva or nasal fluid suggesting they were not the result of swallowed secretions. Nor did they reflect a generalized profile of cytokine and chemokine expression within the gastrointestinal tract, as their levels were distinct from that found within the duodenum. They were thus likely to reflect local mucosal inflammation. Using this methodology we have shown that levels of eotaxin-1 and MCP-4 were significantly greater in children with active EoE than controls. At present, endoscopy is the only validated method of determining the presence of mucosal inflammation and thus disease activity in EoE. As this is an invasive procedure, there has been a previous attempt to measure local expression of inflammatory mediators using an esophageal string technique[4]. This technique demonstrated that levels of eosinophil-derived proteins within mucosal lining fluid are capable of discriminating active EoE from children with disease in remission. The esophageal SAM test has the advantage of providing a profile of inflammatory mediators within the mucosal lining fluid which can be compared to the adjacent underlying mucosa as a way of validating this procedure.

This was a pilot study to determine whether the esophageal SAM test could be used successfully to detect local expression of various cytokines and chemokines. As such, and due to rarity of the disorder, the numbers of children enrolled in our study were small. This is likely therefore to have reduced not only our likelihood of detecting a true effect but also the chance that a statistically significant result reflects a true effect. The results therefore need to be interpreted with caution and require validation from a larger series of individuals. Nevertheless, the profile of cytokines and chemokines within the gastrointestinal and respiratory tracts indicate that certain mediators were ubiquitous whereas others appeared to be more localised. The constitutive expression of mediators at various epithelial surfaces is thought to play an important physiological role in regulating the local innate immune system. However, the profile of local cytokine and chemokine expression within the esophagus of normal children is unknown and how much of the signal seen can be attributed to either atopy or esophageal dysfunction remains to be established.

Our results are consistent with previous findings of increased eotaxin-1 mRNA within the esophagus of children with EoE[8]. Eotaxin-1 is likely to be one of several chemokines involved in eosinophil recruitment and the relative contributions of the different eotaxins in inducing eosinophils to the esophagus at baseline and following allergen exposure remains to be clarified. We did not see a significant difference in eotaxin-3 levels between children with active EoE and controls although median levels were 5-fold higher in children with EoE. Previous studies have shown eotaxin-3 mRNA[3] and protein[1] expression to be highly upregulated in EoE. Our inability to detect a significant

difference between children with active EoE and controls is unlikely to be attributed to the localization of this chemokine within the esophagus. Eotaxin-3 immunoreactivity has been localized to mature squamous cells in children with EoE[8] and thus is likely to be detected within the mucosal lining fluid. It is likely that a significant difference would be apparent in a larger sample of cases and controls. Various chemokines have been implicated in the recruitment and activation of eosinophils following allergen challenge in allergic inflammation. Monocyte chemotactic peptide-4 (MCP-4, CCL-13) is a potent chemoattractant for monocytes and eosinophils and has been shown to exhibit increased expression in both atopic and non-atopic asthmatics[9]. Like eotaxin, MCP-4 acts via CCR3 receptors expressed on eosinophils and is involved in the recruitment of these cells during the late-phase allergic response[10]. A large prospective controlled study of esophageal mucosal inflammatory cytokine responses using in vitro human tissue culture from children with EoE[11] reveals an important role for CD8+ lymphocytes, and suggests that the pathogenesis of EoE is more complex than an isolated eosinophil/Th2 response, involving wider cell types and cytokines within innate immune networks. Indeed it seems likely that in the quest for a biomarker in EoE, a panel of activation rather than a single cytokine will prove most useful.

We conclude that the SAM test can be used to detect locally expressed chemokines and cytokines within the esophageal lining fluid and that eotaxin-1 and MCP-4 may be useful as diagnostic markers in EoE. These data justify further investigation and validation of the esophageal SAM test as a minimally invasive method for diagnosis and monitoring of EoE.

Eleanor Minshall[1] PhD

Harsita Patel[2] MBBS

Nicholas Francis[3] FRCPath

Trevor Hansel[4] FRCPath

John O. Warner[2] FRCPCH

Robert J. Boyle[2] PhD

John Fell[5] FRCPCH

Jenny Epstein[5] PhD

[1]Department of Paediatric Allergy, Cambridge University Hospitals NHS Foundation Trust, [2]Paediatrics and the NIHR Biomedical Research Centre, [3]Histopathology, St Mary's Hospital and [4]The Imperial Clinical Respiratory Research Unit, Imperial Healthcare NHS Trust, [5]Department of Paediatric Gastroenterology, Chelsea and Westminster Hospital, London.

References

1. Dellon ES, Chen X, Miller CR, Woosley JT, Shaheen NJ. Diagnostic utility of major basic protein, eotaxin-3, and leukotriene enzyme staining in eosinophilic esophagitis Am J Gastroenterol 2012; 107:1503–1511.

2. Straumann A, Bauer M, Fischer B, Blaser K, Simon H-U. Idiopathic eosinophilic esophagitis is associated with a TH2-type allergic inflammatory response. J Allergy Clin Immunol 2001;108:954-61.

3. Blanchard C, Wang N, Stringer KF, Mishra A, Fulkerson PC, Abonia JP, et al. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. J Clin Invest 2006; 116:536–547

4. Furuta GT, Kagalwalla AF, Lee JJ, Alumkal P, Maybruck BT, Fillon S, et al. The oesophageal string test: a novel, minimally invasive method measures mucosal inflammation in eosinophilic oesophagitis. Gut 2013;62(10):1395-405.

5. Chawes BL, Edwards MJ, Shamji B, Walker C, Nicholson GC, Tan AJ, et al. A novel method for assessing unchallenged levels of mediators in nasal epithelial lining fluid. J Allergy Clin Immunol. 2010;125(6):1387-1389.

6. Gupta SK, Fitzgerald JF, Kondratyuk T, HogenEsch H. Cytokine expression in normal and inflamed esophageal mucosa: a study into the pathogenesis of allergic eosinophilic esophagitis. J Pediatr Gastroenterol Nutr 2006; 42:22–26.

7. Lucendo AJ, De RL, Comas C, Caballero T, Bellon T. Treatment with topical steroids downregulates IL-5, eotaxin-1/CCL11, and eotaxin-3/CCL26 gene expression in eosinophilic esophagitis. Am J Gastroenterol 2008;103(9):2184–2193.

8. Tantibhaedhyangkul U, Tatevian N, Gilger MA, Major AM, Davis CM. Increased esophageal regulatory T cells and eosinophil characteristics in children with eosinophilic esophagitis and gastroesophageal reflux disease. Ann Clin Lab Sci. 2009;39(2):99-107.

9. Ying S, Meng Q, Zeibecoglou K, Robinson DS, Macfarlane A, Humbert M, et al. Eosinophil chemotactic chemokines (eotaxin, eotaxin-2, RANTES, monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and C-C chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (intrinsic) asthmatics. J Immunol 1999;163(11); 6321-6329.

10. Ying S, Robinson DS, Meng Q, Barata LT, McEuen AR, Buckley MG, et al. C-C chemokines in allergen-induced late-phase cutaneous responses in atopic subjects: association of eotaxin with early 6-hour eosinophils, and of eotaxin-2 and monocyte chemoattractant protein-4 with the later 24-hour tissue eosinophilia, and relationship to basophils and other C-C chemokines (monocyte chemoattractant protein-3 and RANTES). J Immunol. 1999;163(7):3976-84.

11. Sayej WN, Menoret A, Maharjan AS, Fernandez M, Wang Z, Balerezo F, et al. Characterizing the inflammatory response in esophageal mucosal biopsies in children with eosinophilic esophagitis. Clin Transl Immunol 2016;5(7):e88

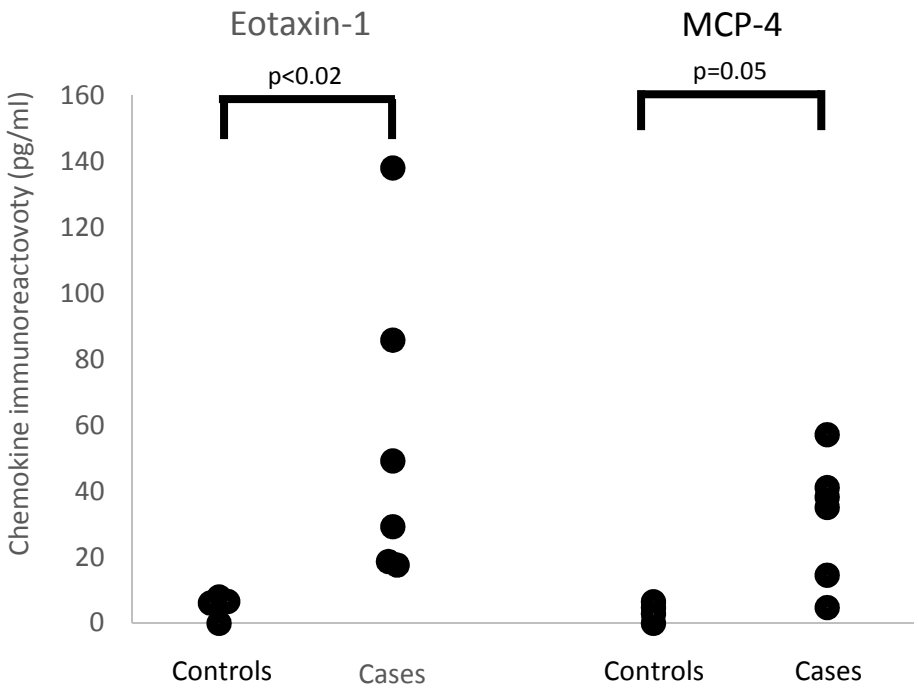

Figure 1. *Eotaxin-1 and MCP-4 immunoreactivity in esophageal lining fluid in active EoE (n = 6) and controls (n = 4). Data were analysed using a Fisher's exact test due to the small sample size.*