Pediatr Pol 2023; 98 (4): 293-299
DOI: https://doi.org/10.5114/polp.2023.133532
Submitted: 2.04.2023, Accepted: 9.07.2023, Published: 11.12.2023

PEDIATRIA Polska
Polish Journal of PAEDIATRICS

ORIGINAL PAPER

Evaluation of MMP-1 and TIMP-1 expression in eosinophilic esophagitis and their usefulness in making therapeutic decisions – a preliminary study

Katarzyna Zdanowicz, Artur Rycyk, Joanna Reszec, Monika Skubis-Zalewska, Dariusz Marek Lebensztejn, Urszula Daniluk

Medical University of Bialystok, Bialystok, Poland

ABSTRACT

Introduction: The incidence of eosinophilic esophagitis (EoE) is rapidly increasing in both children and adults. However, despite extensive research, data on disease pathogenesis and useful markers for predicting treatment response are still lacking. The aim of the study was to analyze matrix metalloproteinase-1 (MMP-1) and tissue inhibitor of metalloproteinase-1 (TIMP-1) expression in the esophagus of pediatric patients with EoE.

Material and methods: This single-center study included newly diagnosed children with EoE based on endoscopic and histopathological results, children with esophageal eosinophilia (EE) not meeting the EoE criteria and healthy controls. Immunohistochemistry for MMP-1 and TIMP-1 was performed.

Results: Among the 30 children whose biopsy specimens were evaluated, 10 patients were diagnosed with EoE, 10 patients with EE and 10 were healthy controls. Weak expression of MMP-1 in esophageal samples was observed in half or more cases in each group. However, strong MMP-1 expression was noted in half of EoE patients before treatment with a decrease in number of cases after treatment. Interestingly, lack of TIMP-1 expression was noted in all EoE patients, in contrast to EE samples, or control cases. Children with strong MMP-1 expression at the time of EoE diagnosis appeared to respond less well to proton pump inhibitors (PPIs) either alone or in combination with an elimination diet. There were no differences in MMP-1 expression with PPI monotherapy as opposed to combination therapy.

Conclusions: Our data revealed that children with EoE did not express TIMP-1 in esophageal tissue, unlike EE and healthy controls. More intense expression of MMP-1 at diagnosis was associated with a poor response to treatment.

KEY WORDS:

children, MMP-1, proton pump inhibitors, TIMP-1, eosinophilic esophagitis.

INTRODUCTION

Eosinophilic esophagitis (EoE) is a chronic, immune/antigen-mediated condition characterized by infiltration of eosinophils in the esophageal mucosa and causing extensive tissue remodeling [1, 2]. Remodeling of the esophagus leads to dysmotility, strictures and rigidity [3]. The diagnosis is confirmed by the histopathological examination of the mucosa biopsies. The minimum number of eosinophils in the esophageal mucosa necessary for the EoE diagnosis is 15 per high-power field (hpf) [1]. Histological changes related to remodeling include epithelial basal zone hyperplasia, dilated intercellular spaces, subepithelial angiogenesis, fibrosis within the lamina propria and smooth muscle hyperplasia [4]. According to the latest guidelines, each type of anti-inflammatory

ADDRESS FOR CORRESPONDENCE:

Katarzyna Zdanowicz, Medical University of Bialystok, Bialystok, Poland, phone: +48 85 745 07 10, e-mail: kazdanowicz@gmail.com

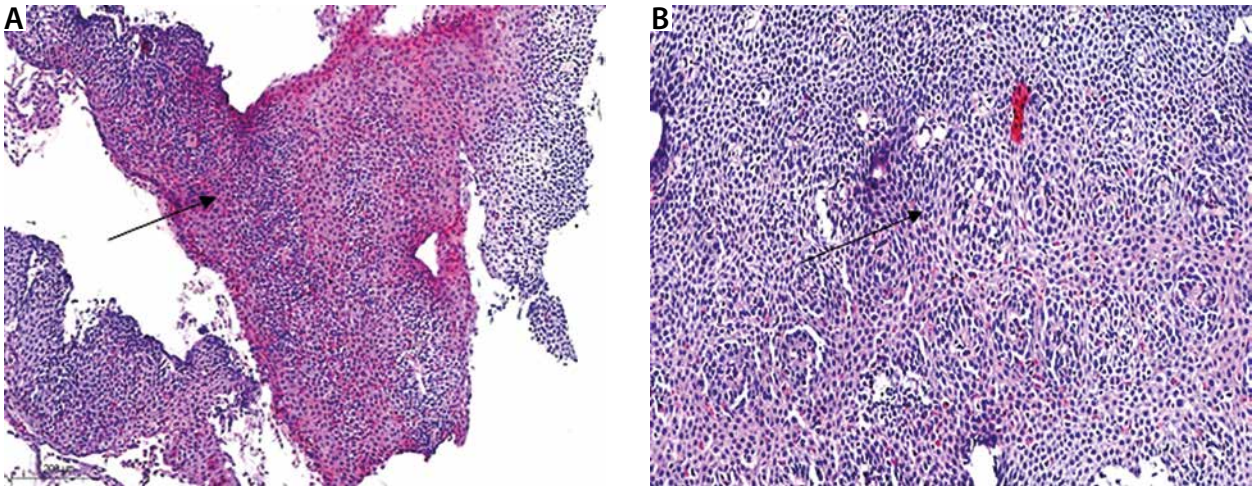

FIGURE 1. A, B) HE staining for eosinophilic esophagitis. Arrow shows the eosinophilic infiltration within squamous epithelium of the esophagus. Magnification 50× (**A**). Eosinophilic esophagitis with numerous eosinophils within mucosa. Magnification 100× (**B**)

therapy (proton pump inhibitors – PPIs, topical steroid) and an elimination diet are the first-line treatment. Despite many studies on the course of EoE, there is still no answer to the question of why not all patients improve after therapy or what criteria should be followed when choosing one of the therapeutic options [5].

In recent years several immunohistochemical markers of inflammation and epithelial integrity have been evaluated in EoE [6]. Among them are matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases, that are involved in extracellular matrix proteolysis, cellular migration, collagen breakdown and angiogenesis [7]. MMP-1 is classified as a fibroblast or interstitial collagenase and is mainly secreted from fibroblasts and macrophages [8]. The activity of MMPs is controlled by tissue inhibitor of metalloproteinases (TIMPs). TIMP-1 is a protein that inhibits MMP-1, MMP-3 and MMP-9. MMPs and TIMPs form reversible complexes in a stoichiometric ratio of 1 : 1 [9]. The balance between MMP-1 and TIMP-1 is substantial for maintaining physiological function, but its dysregulation may induce numerous diseases connected with fibrosis and inflammation [10]. In the course of the inflammatory process, three different situations have been observed: overexpression of MMPs without a simultaneous increase in TIMPs, minimal increase in MMPs with a sudden decrease in TIMPs expression, and a parallel increase in MMPs and a decrease in the level of TIMPs [11]. MMP expression has been evaluated in inflammatory diseases of the gastrointestinal tract, mainly Crohn's disease (CD) and ulcerative colitis (UC) [11, 12]. To date, one study has assessed the expression of MMPs-2 and 14 in patients with EoE [13]. To the best of our knowledge, there are currently no data available on the expression MMP-1 and TIMP-1 in EoE cases.

The aim of our study was to evaluate the immunohistochemical expression of MMP-1 and TIMP-1 in pediatric patients with newly diagnosed EoE in comparison with children diagnosed with esophageal eosinophilia (< 15 eosinophils/hpf) and healthy controls. In addition, we compared the effect of PPI therapy as a single therapeutic strategy or in combination with an elimination diet on expression of these proteins. The next aim of the study was to compare the expression of MMP-1 and TIMP-1 in children with EoE and coexistent allergy and various levels of esophageal eosinophilic infiltration.

MATERIAL AND METHODS

We conducted an analysis of patients of a tertiary Pediatric Teaching Hospital from 2016 to 2019. The study protocol was approved by the local Ethics Committee and conformed to the tenets of the Declaration of Helsinki. A total of 30 patients were included in the study. Of these, 10 children were diagnosed with EoE according to the criteria published in the guidelines in 2017 [1] (Figure 1), 10 children were diagnosed with esophageal eosinophilia (EE) not meeting the histological EoE criteria (< 15 eosinophils/hpf) and 10 children were a control group diagnosed with a functional gastrointestinal disorder with a normal appearing esophagus without eosinophilia (0 eosinophils/hpf). The inclusion criterion in patients with EoE was the lack of obvious fibro-stenotic features at the onset of disease to assess early evidence of fibrous esophageal remodeling at follow-up.

In the group of children with EoE, the data were analyzed from the moment of diagnosis and after the applied treatment. We obtained data from the first and follow-up visits in the gastroenterology outpatient clinic or department. Patients with a prominent eosinophilic infiltrate in gastric or duodenal biopsies, or using PPIs for at least 3 months prior to the first endoscopy, were excluded from the analysis. Patients diagnosed with EoE in a medical facility other than ours were also excluded.

Demographic data including coexisting diseases, presenting symptoms, biopsy results and endoscopic findings were collected from medical records. During the di-

agnosis and follow-up visits for the EoE group, patients/guardians answered standard questions about clinical symptoms, and the answers were included in the patients' medical records. Patients/guardians were asked to rate the severity of symptoms after treatment as "clinical improvement" or "no clinical improvement". "Clinical improvement" was defined as complete symptom resolution written in the medical record, and "no clinical improvement" referred to patients with persistent or worsening symptoms. The results of absolute eosinophil count in blood were collected from the medical record. The diagnosis of allergy was made on the basis of medical records and food sensitization was verified by skin prick tests or allergen-specific serum IgE in all patients.

All children underwent esophagogastroduodenoscopy with esophageal biopsies from 2 or more levels (at least 6 biopsies were taken). The same pathologist analyzed all samples. Doubtful cases were discussed with the second pathologist. Follow-up upper endoscopy with esophageal biopsies was performed in EoE patients after the initial 6- to 12-week course of treatment according to the guidelines [1]. "Endoscopic improvement" was defined as resolution of the lesions or reduction in lesion severity compared to the previous examination which was noted in the patient medical records, and "no endoscopic improvement" was defined as persistence of previously identified abnormalities. "Histological improvement" was defined as peak eosinophil count of < 15 eosinophils/hpf after therapy. All children with EoE were treated with PPIs or PPIs in combination with an elimination diet. The PPI therapy was based on omeprazole (1–2 mg/kg b.w./day). The elimination diet was based on the empiric six-food (milk, egg, soy, wheat, fish, nuts) elimination diet (SFED) or allergy testing-based elimination diet (ATBD).

Expression of MMP-1 and TIMP-1 proteins was assessed by immunohistochemical methods. We used archival formalin-fixed, paraffin-embedded tissue from routine histopathologic work-up, which had been performed under standardized conditions. Slides were cut on a sliding microtome into 4 μm thick sections and were deparaffinized in xylene substitute, rehydrated through three changes of alcohol and subjected to antigen retrieval. Endogenous peroxidase was blocked for 5 minutes. Next, slides were incubated with anti-human antibodies: rabbit polyclonal anti-MMP1 (1 : 200 dilution; ab38929; Abcam) and rabbit polyclonal anti-TIMP-1 (1 : 1000 dilution; ab61224, Abcam) for 1 hour at room temperature. Following the reaction in the streptavidin-biotin system (Biotinylated Secondary Antibody, Streptavidin-HRP, Novocastra, UK) the antigen-antibody complex was visualized using chromogen 3,3-diaminobenzidine (DAB, Novocastra, UK). Slides were counterstained with hematoxylin and evaluated under the light microscope. The intensity of the immunostaining was assessed in 10 random fields under 20× magnification by an independent pathologist who was blind to all clinical information. The tissues used for positive controls were selected according to manufacturers' recommendations. Negative controls were obtained by omitting the primary antibody in immunohistochemical reaction. Expression of MMP-1 and TIMP-1 was assessed semi-quantitatively according to the following scale: ≤ 10% positive cells (–; negative), 11–50% (+; weak reaction), and ≥ 51% positive cells (++; strong reaction) (modified from reference [14]). Due to the small size of individual groups, descriptive statistics were not performed.

Funding source: Medical University of Bialystok, Poland, grant No: B. SUB.23.344.

RESULTS

The demographic data and coexisting diseases at diagnosis in each group are presented in Table 1. Abdominal pain and dysphagia were most common in children with EoE, affecting 60% and 50% of patients, respectively. Among the endoscopic lesions, the most typical lesions in the EoE group were longitudinal furrows (80%) and reduced vascularity (50%). No macroscopic abnormalities were observed in one patient.

Male predominance was evident in each group. There were no significant differences regarding age and gender between the study groups. Allergy was most frequently reported in the EoE group, mainly as food allergy (50%) and rhinoconjunctivitis (70%). Abnormal gastroesophageal reflux in 24-hour esophageal pH monitoring was observed in only four children. Each child with EoE received PPIs for treatment, but in 5 cases an elimination diet was added due to a diagnosed food allergy.

The differences in number of eosinophils in blood and esophageal samples between groups are presented in Table 2.

Most of the patients with EoE (90%) showed blood eosinophilia (> 400 eosinophils/μl) and the highest median absolute eosinophil number compared to other groups. After EoE treatment, a decrease in absolute eosinophil number in blood, as well as median number of eosinophils in esophageal tissue, was observed in this group (Table 2).

The weak expression of MMP-1 in esophageal samples was observed in half or more cases in each group (Table 3, Figure 2). However, strong MMP-1 expression was noted in half of EoE patients before treatment with a decrease in the number of cases after treatment.

Interestingly, lack of TIMP-1 expression was noted in all EoE patients, and 60% of EE samples, compared to only 20% of control cases. Weak TIMP-1 immunohistochemical reaction was found in 40% of children diagnosed with EE and in 80% of controls. No strong expression of TIMP-1 was observed in any of the patients recruited into the study. There was no evidence of fibrosis in any of the biopsies of patients with EoE, EE, or controls.

The second aim of the study was to evaluate whether EoE therapy affects the MMP-1 and TIMP-1 expres-

TABLE 1. Demographic data and coexistent diseases in patients with EoE, EE and controls

Parameter	EoE (≥ 15 eos/hpf) ($n = 10$)	EE (< 15 eos/hpf) ($n = 10$)	Controls (0 eos/hpf) ($n = 10$)
Age [years]	10 (4–15)	13 (8–17)	12 (6–17)
Male, *n* (%)	9 (90)	7 (70)	7 (70)
Allergy, *n* (%)	9 (90)	4 (40)	2 (20)
GERD, *n* (%)	2 (20)	1 (10)	1 (10)
Treatment, *n* (%)			
PPIs	5 (50)	NA	NA
PPIs + elimination diet	5 (50)	NA	NA

EoE – eosinophilic esophagitis, EE – esophageal eosinophilia, GERD – gastroesophageal reflux disease, PPIs – proton pump inhibitors, NA – not applicable, eos – eosinophils.

TABLE 2. Eosinophils in blood and esophageal tissue in EoE, EE and control groups

Parameter	EoE ($n = 10$)		EE ($n = 10$)	Controls ($n = 10$)
	Before treatment	After treatment		
Absolute eosinophil count in blood [cells/µl], median (min.–max.)	650 (160–970)	520 (230-1340)	265 (90–910)	350 (70–1100)
Eosinophilia (> 400 eos/µl), *n* (%)	9 (90.0)	6 (60.0)	1 (10.0)	4 (40.0)
Peak eosinophil [count/hpf], median (min.–max.)	30 (15–45)	23 (8–50)	4 (1–7)	0 (0–0)

EoE – eosinophilic esophagitis, EE – esophageal eosinophilia, hpf – high-power field, NS – not significant.

TABLE 3. Immunohistochemical expression of MMP-1 and TIMP-1 in tissues of EoE (before and after treatment), EE and controls (Figure 2)

Parameter		EoE ($n = 10$)		EE ($n = 10$)	Controls ($n = 10$)
		Before treatment	After treatment		
MMP-1, *n* (%)	0	0 (0)	0 (0)	0 (0)	0 (0)
	+	5 (50)	8 (80)	7 (70)	8 (80)
	++	5 (50)	2 (20)	3 (30)	2 (20)
TIMP-1, *n* (%)	0	10 (100)	10 (100)	6 (60)	2 (20)
	+	0 (0)	0 (0)	4 (40)	8 (80)
	++	0 (0)	0 (0)	0 (0)	0 (0)

EoE – eosinophilic esophagitis, EE – esophageal eosinophilia, MMP-1 – matrix metalloproteinase-1, TIMP-1 – tissue inhibitor of metalloproteinase-1.

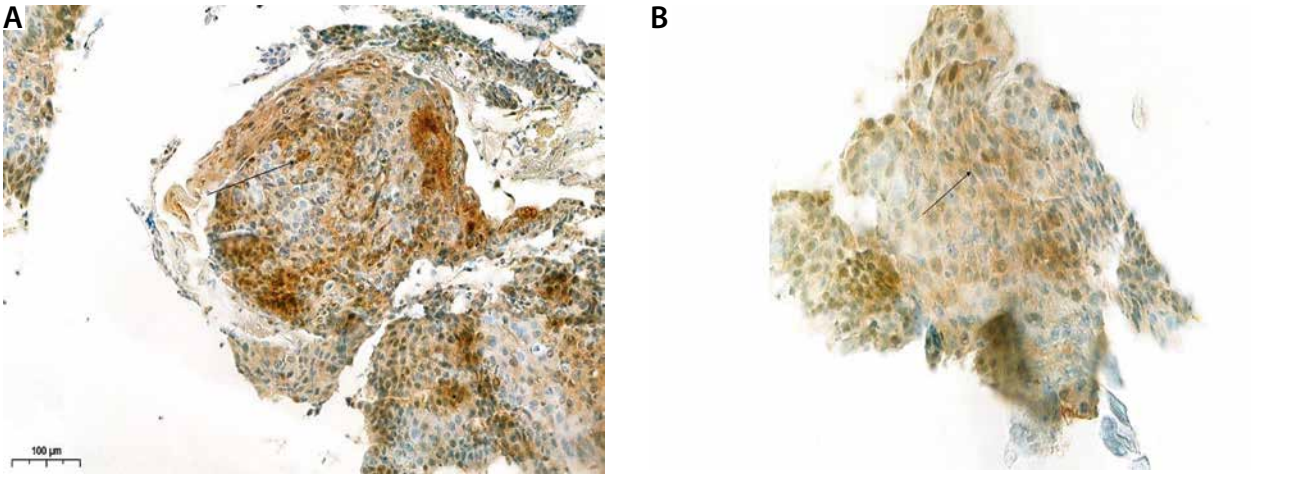

FIGURE 2. A, B) MMP-1 membranous-cytoplasmic strong diffuse staining (++) within eosinophilic esophagitis. Magnification 100× (**A**). MMP-1 membranous-cytoplasmic weak (+) but diffuse staining within eosinophilic esophagitis. Magnification 200× (**B**)

TABLE 4. Association of MMP-1 expression with clinical, endoscopic and histological response to therapy in patients with EoE

Parameter		Clinical improvement		Endoscopic improvement		Histological improvement	
		Yes ($n = 5$)	No ($n = 5$)	Yes ($n = 4$)	No ($n = 6$)	Yes ($n = 4$)	No ($n = 6$)
Expression before treatment							
MMP-1, *n* (%)	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	+	3 (60)	2 (40)	3 (75)	2 (33)	3 (75)	2 (33)
	++	2 (40)	3 (60)	1 (25)	4 (67)	1 (25)	4 (67)
Expression after treatment							
MMP-1, *n* (%)	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	+	4 (80)	4 (80)	3 (75)	5 (83)	3 (75)	5 (83)
	++	1 (20)	1 (20)	1 (25)	1 (17)	1 (25)	1 (17)

EoE – eosinophilic esophagitis, MMP-1 – matrix metalloproteinase-1.

sion. However, due to the lack of TIMP-1 expression in all EoE patients, this protein was not assessed in this part of the study. Association of MMP-1 expression with clinical, endoscopic and histological response in patients with EoE is presented in Table 4. Overall improvement (clinical, endoscopic and histological) was observed in 4 patients. Regardless of clinical, endoscopic and histologic improvement, most cases showed weak MMP-1 expression after treatment. However, among patients with strong expression of MMP-1 at diagnosis of EoE, only 40% of cases demonstrated clinical improvement. Endoscopic and histologic improvement was observed less frequently.

DISCUSSION

Currently, there are no clinical or laboratory factors that could predict the response to treatment of EoE patients; therefore we chose the immunohistochemical method to test whether assessment of MMP-1 or TIMP-1 expression in esophageal biopsies is useful for clinicians in everyday practice. To our knowledge, no study that evaluated the expression of MMP-1 and TIMP-1 in patients with EoE, both in children and adults, has been published so far.

We found that strong MMP-1 expression was more common in patients with EoE. At the same time, TIMP-1 expression was not detected in any of the EoE cases. Interestingly, the fewer eosinophils were found in esophageal tissue, the greater was the TIMP-1 expression there. Among our EE patients, less than half of them showed weak expression of TIMP-1, in contrast to the healthy subjects, where the proportion of patients with strong expression of TIMP-1 predominated. The only study investigating the relationship of TIMP-1 expression with eosinophilic infiltration expressed as eosinophilic cationic protein (ECP) concentration was performed by Lee *et al.* in the tissues of nasal polyps [15]. No statistically significant correlation was found between TIMP-1 and ECP. However, a relationship between MMPs and the migration of eosinophils was found in patients with allergic rhinitis, showing a strong correlation between MMP-13 and the number of eosinophils in nasal mucosa specimens [16]. This may suggest the role of MMPs in eosinophil recruitment and migration. In our study, no significant correlations were found between the eosinophilic infiltrate and the expression of MMP-1 in the esophagus, and the difference in the expression of MMP-1 or TIMP-1 depending on the incidence of allergy or other type of esophagitis (GERD).

So far, the evaluation of MMP-1 and TIMP-1 expression in esophageal samples has been associated with Barrett's esophagus and esophageal adenocarcinoma in adult patients [8, 17, 18]. MMP-1 was expressed in almost 35% of the samples with Barrett's esophagus and esophageal adenocarcinoma without significant differences in expression between patients [17]. However, the expression of TIMP-1 was associated with a more aggressive disease course in patients with Barrett's esophageal adenocarcinoma [8]. On the other hand, another study showed lower TIMP-1 expression in node-positive esophageal adenocarcinoma patients compared to patients without lymph node involvement [18]. Perhaps the expression of MMP-1 or TIMP-1 in the esophagus depends on the type of infiltrating cells.

The available data on the detection of MMPs in children with esophageal diseases concern MMP-2 and MMP-14 [13, 19]. Pilmane *et al.* observed in children with esophageal atresia increased MMP-2 expression in the distal part and decreased MMP-2 expression in the proximal part of the esophagus compared to the control group [19]. Beppu *et al.* found elevated expression of MMP-2 and MMP-14 in in the esophagus samples of pediatric patients with EoE. Furthermore, epithelial expression of MMP-14 was positively correlated with the degree of fibrosis of the lamina propria. An important observation of this study was the effect of topical steroids on the expression of MMPs. Reduced expression of MMP-2 and MMP-14 was found in patients with simultaneous clinical, endoscopic and histological im-

provement after budesonide/fluticasone therapy [13]. On the other hand, in our study most children with strong MMP-1 expression before treatment with PPIs, either alone or in combination with an elimination diet, did not show a clinical, endoscopic and histological response to such therapy. It seems that the assessment of MMP-1 expression may be a useful tool in making therapeutic decisions in children with EoE; however, more research is needed to confirm such a hypothesis. Based on our results, changes in MMP-1 expression after a few weeks of treatment did not reflect treatment efficacy. However, due to the short period of observation and the small study group, we are not able to confirm whether the changes observed in the immunohistochemical analysis precede a positive response to treatment; more research is needed. MMP-1 seems to play an important role in the degradation of type I collagen, and TIMP-1 participates in the regulation of MMP activity levels [20, 21]. Increase in MMP-1 and decrease in TIMP-1 expression were observed in animal models with pulmonary fibrosis [21]. In our study we found enhanced expression of MMP-1 combined with a lack of expression of its inhibitor TIMP-1 in the esophagus, but no evidence of fibrosis. Given that esophageal fibrosis is important in the course of EoE, it would be interesting to evaluate the relationship between MMP-1 expression and collagen deposition in EoE patients in the long-term follow-up, especially in those with strong expression of MMP-1 at the onset of the disease.

The strength of our study is that we were the first to evaluate the expression of MMP-1 and TIMP-1 in EoE and to analyze the influence of the applied treatment on the immunohistochemical expression of these proteins. We also assessed the relationship of the change in MMP-1 and TIMP-1 expression with the clinical, endoscopic and histological response. There are a few limitations to our study. The most important of these is the small number of enrolled patients, due to the small number of diagnosed children. First, the small number of participants did not allow us to generalize our results. Our study was designed as an explanatory study to generate only pathophysiological theories of disease. The number of patients enrolled in the study was low due to the time span and the monocentric nature of the study. We understand that our results may be subject to errors of omission (type II error), and we did not interpret non-significant statistical results as underlying a true lack of differences. Another limitation is the lack of patients receiving topical steroids in the study, and thus the inability to assess their effect on the expression of MMP-1 and TIMP-1.

CONCLUSIONS

Our data revealed that children with EoE did not express TIMP-1 in esophageal tissue, unlike EE and healthy controls. However, it was found that stronger expression of MMP-1 at diagnosis was associated with poor response to treatment. More research is needed to assess its utility as a tool in making therapeutic decisions in daily practice.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Lucendo AJ, Molina-Infante J, Arias Á, et al. Guidelines on eosinophilic esophagitis: evidence-based statements and recommendations for diagnosis and management in children and adults. United European Gastroenterol J 2017; 5: 335-358.
2. Aceves SS, Newbury RO, Dohil R, et al. Distinguishing eosinophilic esophagitis in pediatric patients: clinical, endoscopic, and histologic features of an emerging disorder. J Clin Gastroenterol 2007; 41: 252-256.
3. Hassan M, Aceves S, Dohil R, et al. Esophageal compliance quantifies epithelial remodeling in pediatric patients with eosinophilic esophagitis. J Pediatr Gastroenterol Nutr 2019; 68: 559-565.
4. Aceves SS, Ackerman SJ. Relationships between eosinophilic inflammation, tissue remodeling, and fibrosis in eosinophilic esophagitis. Immunol Allergy Clin North Am 2009; 29: 197-211, xiii-xiv.
5. Zdanowicz K, Kucharska M, Lebensztejn D, et al. Treatment effectiveness in paediatric patients with eosinophilic oesophagitis. Pediatr Pol 2022; 97: 221-228.
6. Zdanowicz K, Kucharska M, Reszec J, et al. Immunohistochemical markers for eosinophilic esophagitis. Scand J Gastroenterol 2020; 55: 1277-1283.
7. Bassiouni W, Ali MAM, Schulz R. Multifunctional intracellular matrix metalloproteinases: implications in disease. FEBS J 2021; 288: 7162-7182.
8. Grimm M, Lazariotou M, Kircher S, et al. MMP-1 is a (pre-)invasive factor in Barrett-associated esophageal adenocarcinomas and is associated with positive lymph node status. J Transl Med 2010; 8: 99.
9. Gomez DE, Alonso DF, Yoshiji H, et al. Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. Eur J Cell Biol 1997; 74: 111-122.
10. Grünwald B, Schoeps B, Krüger A. Recognizing the molecular multifunctionality and interactome of TIMP-1. Trends Cell Biol 2019; 29: 6-19.
11. Marônek M, Marafini I, Gardlík R, et al. Metalloproteinases in inflammatory bowel diseases. J Inflamm Res 2021; 14: 1029-1041.
12. Daniluk U, Daniluk J, Guzinska-Ustymowicz K, et al. Usefulness of metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in clinical characterisation of children with newly diagnosed Crohn's disease. J Paediatr Child Health 2020; 56: 1233-1241.
13. Beppu L, Yang T, Luk M, et al. MMPs-2 and -14 are elevated in eosinophilic esophagitis and reduced following topical corticosteroid therapy. J Pediatr Gastroenterol Nutr 2015; 61: 194-199.
14. Matoso A, Mukkada VA, Lu S, et al. Expression microarray analysis identifies novel epithelial-derived protein markers in eosinophilic esophagitis. Mod Pathol 2013; 26: 665-676.
15. Lee YM, Kim SS, Kim HA, et al. Eosinophil inflammation of nasal polyp tissue: relationships with matrix metalloproteinases, tissue inhibitor of metalloproteinase-1, and transforming growth factor-beta1. J Korean Med Sci 2003; 18: 97-102.
16. Mori S, Pawankar R, Ozu C, et al. Expression and roles of MMP-2, MMP-9, MMP-13, TIMP-1, and TIMP-2 in allergic nasal mucosa. Allergy Asthma Immunol Res 2012; 4: 231-239.
17. García-Varona A, Fernández-Vega I, Santos-Juanes J. Immunohistochemical expression analysis of MMP-1, TIMP-2 and p53 in

Barrett's esophagus, dysplasia and esophageal adenocarcinoma. Pol J Pathol 2021; 72: 48-56.
18. Vegh I, Santiuste AD, Colina F, et al. Relationship between biomarker expression and allelic alteration in esophageal carcinoma. J Gastroenterol Hepatol 2007; 22: 2303-2309.
19. Pilmane M, Ozoliņa L, Ābola Z, et al. Growth factors, their receptors, neuropeptide-containing innervation, and matrix metalloproteinases in the proximal and distal ends of the esophagus in children with esophageal atresia. Medicina (Kaunas) 2011; 47: 453-460.
20. Diniz-Fernandes T, Godoy-Santos AL, Santos MC, et al. Matrix metalloproteinase-1 (MMP-1) and (MMP-8) gene polymorphisms promote increase and remodeling of the collagen III and V in posterior tibial tendinopathy. Histol Histopathol 2018; 33: 929-936.
21. Hu GX, Yao ST, Zeng LH, et al. Effects of hydroxycamptothecin on the expression of matrix metalloproteinase-1 (MMP-1), tissue inhibitor of MMP-1, and type I collagen in rats with pulmonary fibrosis. Genet Mol Res 2015; 14: 4625-4632.