ANDES PEDIATRICA
REVISTA CHILENA DE PEDIATRÍA
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Andes pediatr. 2021;92(5):683-689
DOI: 10.32641/andespediatr.v92i5.2552

ORIGINAL ARTICLE

Normal values of eosinophils in gastric and duodenal mucosa of children referred to upper gastrointestinal endoscopy

Valores de normalidad de eosinófilos en mucosa gástrica y duodenal de niños referidos a endoscopía digestiva alta

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Received: September 25, 2020; Approved: March16, 2021

What do we know about the subject matter of this study?

There is a lack of large studies in the pediatric population regarding a cut-off point of eosinophils (Eos) in gastric and duodenal mucosa in healthy individuals. Eos counts have been reported in biopsies in the northern hemisphere, but in the Latin American population, they have been scarcely explored.

What does this study contribute to what is already known?

It describes in Latin American (Chilean) pediatric population, an Eos count (mean ±1 SD) of 1.06 ± 1.79 Eos/HPF (high power field) in gastric body (n = 27), 1.13 ± 1.79 Eos/HPF in gastric antrum (n = 72), and 10.44 ± 7.09 Eos/HPF in duodenum (n = 30).

Abstract

With the increasing incidence of food allergies, the presence of eosinophils (Eos) in the gastrointestinal mucosa has received increased attention, particularly in the esophagus and colon. However, normal values for the Eos count in the stomach and duodenum in pediatric patients are still limited. The objective of this study was to estimate Eos reference values in stomach and duodenal biopsies of children referred to upper gastrointestinal endoscopy. **Patients and Methods**: Cross-sectional study of biopsies from symptomatic children referred to upper gastrointestinal endoscopy. The endoscopic report, Rapid Urease Test for the presence of *H. pylori*, and the quantitative histological evaluation

Keywords:
Food Allergies;
Eosinophils;
Gastric Mucosa;
Stomach;
Helicobacter pylori

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How to cite this article: Andes pediatr. 2021;92(5):683-689. DOI: 10.32641/andespediatr.v92i5.2552

(number of cells/HFP, high power field) were analyzed. The Eos distribution is described as mean and standard deviation, and also as percentiles since the counts did not have a normal distribution. Statistical analysis included χ^2 test, Wilcoxon test, analysis of variance, and linear regression curves were evaluated as appropriate. **Results**: Of the 170 patients referred to endoscopy, 72 met "normal" criteria (normal endoscopy in macroscopic analysis, negative Rapid Urease Test, and normal biopsy). The median age was 11 years (range 4-16), and 68% were girls. The Eos count (mean ± 1SD) in gastric antrum (n = 72) was 1.13 ± 1.79 Eos/HPF; in gastric body (n = 27), 1.06 ± 1.79 Eos/HPF; and in duodenum (n = 30), 10.44 ± 7.09 Eos/HPF. There were no significant differences by age and sex, or by *H. pylori* infection (p = 0.095). **Conclusions**: We propose an Eos count of 0-3 Eos/HPF for the gastric body, 0-3 Eos/HPF in the antrum, and 3-17 Eos/HPF in the duodenum as a normal range for gastric mucosa in children. This study suggests that in areas with a high prevalence of *H. pylori* infection, the count of Eos does not seem to be a distinctive element and that Eos are commonly present in the gastroduodenal mucosa.

Introduction

The gastrointestinal (GI) tract has more surface area than any other organ in the body and contains the greatest number of immune cells and their products. Eosinophils (Eos) are normally present throughout the GI tract[1], except in the esophagus[2], and there is an upward gradient in the Eos count from cephalic to caudal, with higher levels in the cecum than in the stomach[3,4]. Eosinophils are multifunctional granulocytes involved in allergies, parasitic infections, tumor-host interactions[5,6], and help to maintain intestinal epithelial homeostasis[2,4,7]. Eos may interact by regulating the intestinal microbiota, especially by the antibacterial effect of their granular proteins and the ability to modify innate immunity[5,8].

In recent decades, the role of Eos has been studied, specifically their role in the maintenance of health or the development of diseases of the GI tract. Eosinophil-associated gastrointestinal disorders (EGIDs) include several diseases characterized by eosinophilic inflammation in different segments of the GI tract if no other known causes of eosinophilia are identified[9]. EGIDs include eosinophilic esophagitis (EoE), eosinophilic gastritis (EoG), eosinophilic gastroenteritis (EoGE), and eosinophilic colitis (EoC)[4,8,10]. However, most of the known information refers to EoE[11,12] while the remaining EGIDs are considered very infrequent[13,14]. Therefore, despite technological advances, diagnostic criteria for several EGIDs have not yet been clearly established[15-21].

Compared with EoE, eosinophilic gastritis is a very rare entity. There is a lack of large studies in the pediatric population and there is no consensus on a cut-off point for Eos count in gastric and duodenal mucosa in healthy individuals[4,9], even less in the pediatric population[6,7,22-24]. In the available studies, there is much variability in the threshold of gastric Eos count considered as diagnostic by the different authors, from 20 Eos/HPF, 30 Eos in at least 5 HPF, to some that consider 80 Eos/HPF[8].

From the information available to date, regarding normal values of Eos in gastric mucosa, there have been reports of Eos counts in biopsies in the pediatric population of the northern hemisphere[15,16,21], whereas there has been little exploration in the Latin American population[11].

The objective of our study was to estimate reference values of eosinophils in stomach and duodenum biopsies from Chilean children referred to upper GI endoscopy (EGD) due to common digestive symptoms (chronic abdominal pain, CAP), whose macroscopic and histological findings were normal.

Patients and Method

We used histological analyses from 2 databases of previous studies, both of cross-sectional design, with similar inclusion and exclusion criteria (patients under 18 years of age, referred to upper endoscopy, mostly due to CAP). From both databases, we selected those patients that their endoscopic report was available, whose results were categorized as normal, gastropathy, antral nodularity, duodenopathy, peptic ulcer disease, among others; the result of the rapid urease test to determine the presence of *Helicobacter pylori* (*H. pylori*) (positive or negative), and the histopathological analysis.

Eosinophil count

Biopsy samples were fixed with 10% v/v (volume/volume) buffered formalin with pH 7.4 for 24 to 36 hours and subsequently processed with routine histological techniques to embed them in paraffin in order to obtain tissue blocks. These blocks were cut 5 µm thick in a rotary microtome to obtain histological sections, which were subsequently deparaffinized and

stained with hematoxylin and eosin (HE). After this processing, the sections were analyzed by light microscopy.

An anatomopathologist performed a quantitative evaluation of Eos in the mucosa (cells/HPF) considering as a "high power field" that with a magnification of 400x, equivalent to 0.237 mm^2, in each area (antrum, body, and duodenum, when available), considering "satisfactory" those samples that met the following criteria:

- Contain at least 75% of the field with cells.
- After the sliding process, they allowed the evaluation of at least 5 HPF.
- It was possible to recognize the characteristics of the antrum, gastric body, or duodenum, depending on the case.

Eos were considered as those in which the nucleus was recognized, associated with eosinophilic granules grouped around it (Figure 1). The eosinophil count was performed from the mucosa of the digestive tissue at the lamina propria level.

The pathologist performed a blinded process regarding the clinical data of these children and to the presence of *H. pylori* by rapid urease test.

Data analysis

For the statistical analysis, the data were tabulated into an Excel database and analyzed with the STATA 12 software. The following were evaluated: i) Eos count in the gastric body, antrum, and duodenum, ii) Eos count according to endoscopy results, and iii) for those endoscopies without pathological findings (normal EGD, *H. pylori* by urease test negative, and normal biopsy), the Eos count was evaluated according to the age and sex of the patient. For each patient, the number of Eos per site was considered as the average for at least 5 HPF. The average count and 1 standard deviation (SD) were calculated according to other publications. However, since the variables age and Eos count did not have a normal distribution, the description of the percentiles 5-25-50 (median)-75-95 was included.

The analysis of differences between qualitative variables was performed with $\chi 2$ test, and quantitative parameters were compared using the Wilcoxon test. Analysis of variance was performed for the study between qualitative and quantitative variables. Linear regression curves were also performed for the study between quantitative variables (Eos count in the different tracts according to age).

Ethical Aspects

Both the endoscopic reports and the samples analyzed for this study were obtained in research projects carried out and previously approved by the Ethics Committee of the Faculty of Medicine of the Pontificia Universidad Católica de Chile (Nº 0145-05 and Nº 09-099), along with their respective informed consents. Access to the database was coded and anonymized for the personal data of the participants.

Results

Patients

Biopsies from 170 patients were analyzed. The age had no normal distribution, with a median of 12 years (range 3-16 years), of which 99 were girls (58.2%). There were 72 biopsies available for gastric body analysis, 170 for gastric antrum analysis, and 72 samples for second duodenal portion analysis. Of these samples, 66 samples from the gastric body, 167 from the antrum, and 68 from the duodenum were considered satisfactory.

Eosinophil count in the pediatric population with normal EGD, normal biopsy, and negative rapid urease test for *H. pylori* detection

Of the 170 patients referred for EGD, 72 met the criteria for normality. In this group, the median age was 11 years (range 4-16), 49 were female (68%). Eosinophil count did not show normal distribution (Shapiro-Wilk test, $p < 0.001$). The mean ± SD count in the gastric body (27 samples) was 1.06 ± 1.79 Eos/HPF, in the gastric antrum (71 samples) was 1.13 ± 1.79 Eos/HPF, and in the duodenum (30 samples) was 10.44 ± 7.09 Eos/HPF. Table 1 shows the percentile distribution of the "normal" group. If we consider the 95th percentile

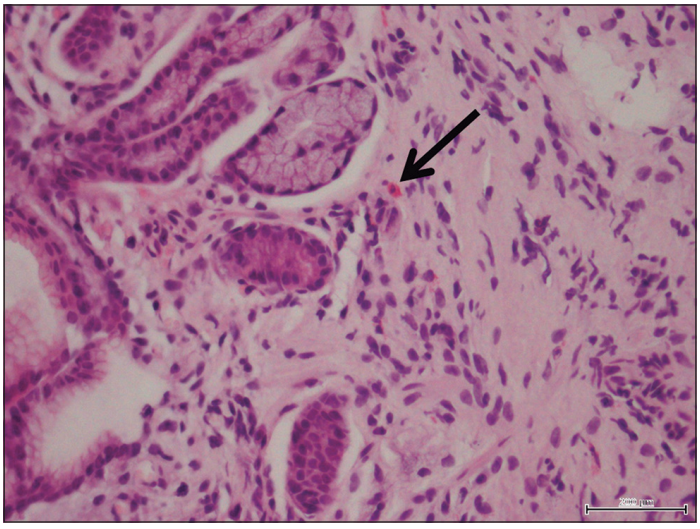

Figure 1. Example of high power field (HPF, 400x) for eosinophil count in gastric mucosa. The arrow indicates an eosinophil, recognizing its nucleus and cytoplasm with an accumulation of eosinophilic granules around it.

as the upper limit of normality, the limit values were 2.7, 5, and 22.5, for the gastric body, gastric antrum, and duodenum, respectively (Table 1).

When analyzed by sex and age, there were no significant differences.

Eosinophil count in the pediatric population with altered EGD

Of the 170 patients referred for EGD, 76 presented some macroscopic alteration. In this group of patients, the median age was 13 years (range 3-16), 37 girls (48.7%). Table 2 shows the details of the macroscopic findings in the altered EGD, with the respective Eos/HPF count.

Eosinophil count in pediatric population according to rapid urease test

Of the total sample, 54 patients had a positive urease test (31.8%). No significant difference was observed for Eos/HPF count in the gastric antrum, gastric body, or duodenum between the population with positive and negative urease tests (p = 0.095) (Table 3).

Discussion

In this study, children with EGD and normal biopsies and negative rapid urease test presented an average eosinophil count in gastric mucosa of 1.06 ± 1.79 Eos/HPF (5-95 percentile: 0-2.7); in gastric antrum 1.13 ± 1.79 Eos/HPF (5-95 percentile: 0-5), and in duodenum 10.44±7.09 Eos/HPF (5-95 percentile: 2.6-22.5). These data are similar to that of other study groups in pediatric populations, such as DeBrosse et al[21], Lowichik et al[15], and Kalach et al[16], although less than that of Chernetsova et al, who used different staining and definition of HPF[25]. In the study by DeBrosse et al., which is one of the initial and most cited studies, biopsies of the GI tract from 28 children in the USA, previously considered as "without diagnostic abnormalities", were re-evaluated and showed 1.9 ± 1.3 and 2.1 ± 2.4 Eos/HPF ± SD in the lamina propria of the antrum and gastric fundus, respectively, and 9.6 ± 5.3 Eos/HPF ± SD in the duodenum[21].

Table 1. Percentile distribution of eosinophil count in the studied sample, in patients with normal endoscopy, normal urease test and normal biopsy.

	Percentile	Normal endoscopy, normal urease test and normal biopsy group, Eos/HPF
Body	p5	0
	p25	0
	p50	0.6
	p75	1.3
	p95	2.7
Antrum	p5	0
	p25	0
	p50	0.6
	p75	1.4
	p95	5
Duodenum	p5	2.6
	p25	5.4
	p50	8.6
	p75	13.5
		22.5

Eos/HPF= eosinofilos/High power field.

In Chile, the information available in the pediatric population is from a study of food allergy markers[26]. 74.1% of patients without food hypersensitivity (FH) presented Eos in the gastric mucosa and 100% of the patients in the group with FH, showing a significant difference, but this difference was observed in the eosinophil count between both groups (no FH group 0.3 ± 0.4 Eos/HPF; FH group 0.63 ± 1.18 Eos/HPF).

Our findings, which are relevant to a previously unanalyzed Latino pediatric population, confirm the appearance of a gradient with an increase in Eos count in the digestive mucosa from proximal to distal. In parallel, this could be attributed to food digestion, in which from cephalic to caudal the contact of the GI mucosa with increasingly digested products increases, also increasing the intraluminal density of bacteria[16].

We found no sex or age differences in Eos counts, like those described by Lowichik et al., DeBrosse et al., and Lwin et al[17].

On the other hand, in our country, the high seroprevalence of *H. pylori* in the adult Chilean population stands out, with 73% positivity[27]. In Chilean children, the prevalence varies from 18.1%[28] to 55.9% depending on the socioeconomic level evaluated[29]. In our study, no association was detected between Eos count and *H. pylori*, similar to that reported by Lwin et al. However, this is different from that described by Kalach et al. and Ashorn et al.[30] and other studies in adults[6].

Recently, in a pediatric study in South Korea[31,32], Eos counts were performed in gastric, duodenal, and colonic mucosa, finding a significant difference, with higher counts in gastric antrum and duodenum in the population with functional disorders of the GI tract according to Rome III classification 2016 study, with 105 patients, and subsequent update to Rome IV in 2018 with 56 patients, suggesting a role of these cells in the pathophysiology of functional disorders, which was not evaluated in this work.

Our study and those previously cited share limitations, since they include children with digestive symptoms referred to EGD (most of them performed for the

Table 2. Distribution of the eosinophil count in the studied sample, in patients with altered endoscopy.

Endoscopic finding	Patients n	Body			Antrum			Duodenum		
		Mean ±SD	Median (p25-75)	n*	Mean ±SD	Median (p25-75)	n*	Mean ±SD	Median (p25-75)	n*
Gastropathy	11	1 ± 0.70	0.7 (0.6-1.2)	6	2.7 ± 2.8	1.4 (0.7-3.8)	11	9.0 ± 4.9	7.4 (6.9-11.2)	6
Antral nodularity	30	2.4 ± 2.0	1.9 (1.1-3)	12	2.2 ± 3.8	0.7 (0.2-2.7)	30	8 ± 4.9	7 (4.8-12.5)	11
Duodenopathy	7	3.8 ± 3.0	4.3 (0.6-6.6)	3	1.7 ± 3.3	0.2 (0.1-1.1)	7	7.0 ± 2.4	7.4 (4.4-9.2)	3
Gastroduodenal ulcer	11	NA		0	2.2 ± 2.3	1.2 (0.5-3.2)	11	NA		0
Other diagnoses	17	1.3 ± 2.0	0.6 (0.1-1.7)	8	1.8 ± 1.6	1.2 (0.8-2.2)	17	20.5 ± 33	10.7 (6.6-12.8)	10
Total	76			29			76			30

n*= number of biopsies with satisfactory sample. n= number of patients.SD= Standard deviation. p= percentile. NA= not available.

Table 3. Comparison of eosinophil count in gastric antrum according to urease test (absence / presence of H. pylori).

Group	Frequency (patients with analizable antrum samples)	Mean Eos/HPF ± SD
NEGATIVE urease test (absence of H. pylori)	113	1.50 ± 2.17
POSITIVE urease test (presence of H. pylori)	54	2.05 ± 3.12
TOTAL	167	1.68 ± 2.52

Eos/HPF= eosinophils/high power field. SD= Standard deviation.

study of CAP), which hinders the use of the term "normality" and therefore, in this study we refer to it as "reference". This study was based on biopsies of children with CAP, with normal macroscopic and microscopic findings. Probably, this group of patients is the closest to what we could call "healthy or normal children". Performing histological studies in the GI tract has ethical limitations in a healthy pediatric population since invasive methods are used for this purpose.

In some studies, it is mentioned that the use only of hematoxylin and eosin (HE) for the staining of the samples would be a limitation for the histological analysis, since it could underestimate the number of Eos present in each analysis[16], proposing the use of Luna staining for eosinophil granules, which is a specific histochemical technique for Eos granules that improve the histological detection of these granulocytes. However, most of the experiences across the globe have been performed with HE staining, with fairly consistent results, as detailed above.

In conclusion, we propose as a reference range for gastric mucosa in children, a body Eos count of 0-3 Eos/HPF, an antrum Eos count of 0-3 Eos/HPF, and duodenum Eos count of 3-17 Eos/HPF. Eos are normally present in the gastroduodenal mucosa. The establishment of reference cut-off points, at early ages, universally validated in different geographical areas, is a necessity for the clinician, and this work is a significant contribution to this area of growing development.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

Financial Disclosure

Fondecyt Project N°1130387, Chile.

Aknowledgments

Diego Romero Vera, Biobank and Projects Manager, Pathologycal Anatomy Department, Faculty of Medicine, Pontificia Universidad Católica de Chile.

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