Original Article

Eosinophil counts in colonic tissue eosinophilia: Investigating specificity and sensitivity of cutoff points and comparing two counting methods

Heyam A. Awad, Yousef E. Abu Osba, Mohammad A. Shaheen, Ammar R. Sfeira

Department of Histopathology, Microbiology and Forensic Medicine, School of Medicine, University of Jordan, Amman, Jordan

Abstract

Background/Aims: The aim of this study was to investigate the specificity and sensitivity of eosinophil cutoff points defining the colonic tissue eosinophilia (TE) and compare the yield of reporting the highest count versus the mean of five high-power fields (HPFs).

Materials and Methods: One hundred and seventy-one cases of colonic TE, including 22 primary eosinophilic colitis (PEC) cases, were compared to one hundred and twenty-one normal controls in the University of Jordan. The highest eosinophil count (EC) and the mean of five HPFs were recorded. The receiver operating characteristic curve (ROC) analysis was used to find the cutoff point with the best sensitivity and specificity.

Results: There was no significant advantage of counting five fields over counting the most densely populated HPF. Using 30 eosinophils per HPF achieved 80% sensitivity and 65% specificity. This point is close to the mean in normal controls plus one standard deviation (SD) (29 per HPF). However, there was overlap between normal counts and TE, using 30 as a cutoff point resulted in 35% false-positive rate. There was no reliable cutoff point to differentiate PEC from secondary TE.

Conclusion: We recommend reporting the highest EC in colonic biopsies and using 30 as a cutoff point, bearing in mind the overlap with normal and correlating with the clinical team to not treat asymptomatic patients. Clinicopathological correlation is essential to separate PEC from secondary TE.

Keywords: Colon, eosinophils, primary eosinophilic colitis, tissue eosinophilia

Address for correspondence: Dr. Heyam A. Awad, Department of Histopathology, Microbiology and Forensic Medicine, School of Medicine, University of Jordan, Amman 1194, Jordan.
E-mail: heyamawad2000@yahoo.com

INTRODUCTION

Colonic tissue eosinophilia (TE) is defined as increased eosinophils within colonic biopsies. It is divided into primary eosinophilic colitis (PEC) and secondary eosinophilia caused by drugs, parasites, inflammatory bowel disease (IBD), and other conditions.[1-3] Recently, TE has gained attention, which is reflected by the increased publications about the subject since 2000.[4] However, a quantitative definition and consensus criteria to diagnose TE are lacking.[5-7] The main reason behind this is the scarcity of published data regarding the normal number of eosinophils. DeBrosse examined eosinophil counts (ECs) in 44 children autopsy specimens.[8] Occasionally, other publications investigated normal eosinophils among children.[5,9-11] Normal ECs have not been adequately investigated among adults. The largest study to date was

Access this article online	
Quick Response Code:	Website: www.saudijgastro.com
	DOI: 10.4103/sjg.SJG_381_19

How to cite this article: Awad HA, Abu Osba YE, Shaheen MA, Sfeira AR. Eosinophil counts in colonic tissue eosinophilia: Investigating specificity and sensitivity of cutoff points and comparing two counting methods. Saudi J Gastroenterol 2020;26:39-45.

conducted by Turner, which included 159 normal and 194 PEC cases.[12] Matsustisha investigated normal counts among Japanese adults.[13] A major limitation of these studies, as pointed by a recent meta-analysis[14] is that cases of functional gastrointestinal (GI) disorders were not excluded from the normal controls, despite the suggested role of eosinophils in these disorders.[15]

Due to the lack of an agreed definition, researchers use variable cutoff points to diagnose TE. In clinical practice, 20 eosinophils/HPF is used.[4,15,16] However, Alhmoud used 50, which is the maximum number reported in normal biopsies,[17] whereas Collin suggested using twice the maximum of normal counts.[2] No previous study attempted to investigate the sensitivity and specificity of these cutoff points.

There are no known histopathological features to separate PEC from secondary TE. Diagnosing PEC is made on clinicopathological grounds, where, TE is reported in symptomatic patients for whom secondary causes are excluded.[18]

Another practical problem that needs to be addressed is to investigate if there are differences regarding reporting the number of eosinophils in the HPF with the highest eosinophil density compared with calculating the mean of eosinophils in several HPFs. Some researchers were content with one HPF[2,17] others counted four[5] or five[12] fields. One study found no statistical difference between counting 3 and 10 fields.[19]

In view of the above mentioned gaps in the literature, this study aims at:

1. Finding a reasonably sensitive and specific cutoff point of abnormal eosinophils
2. Comparing the yield of two counting methods: highest count versus mean of five fields
3. Investigating if there are histopathological features that distinguish PEC from secondary TE.

MATERIALS AND METHODS

This retrospective, cohort study covered the period between January 1, 2016 and August 1, 2018. The computerized system in the histopathology department at the Jordan University Hospital (JUH) was searched for five diagnostic categories in colonic biopsies, including normal mucosa, tissue eosinophilia, drug reaction, ulcerative colitis (UC), and Crohn's disease (CD).

Cases diagnosed as TE (130 cases) were reviewed to separate the PEC from secondary TE. The review included referring to the computerized clinical records to check for drug history, parasitic infections, and other causes of TE. These cases were also discussed at the clinicopathological meeting held at the JUH's Gastrointestinal Unit. Of the 130 cases, 22 were diagnosed as PEC. Of the above 130 cases, 7 were found to have drug-induced TE. A search for "drug effect" and "drug reaction" revealed another five cases. Of these 12 cases, 8 were because of nonsteroidal anti-inflammatory drugs (NSAIDs) and four because of colchicine. A total of 61 CD and 76 UC were included.

In the four categories, the inclusion criteria included documented TE in the histopathological reports and definite clinical diagnosis of the underlying cause after discussion at the clinicopathological meeting. Histological review of the slides included assessing crypt architectural changes and basal plasmacytosis as histological indicators of IBD. All IBD cases included in the study (UC and CD cases) were confirmed as such by the histopathological assessment and clinical evaluation. None of the other TE cases showed architectural abnormality or basal plasmacytosis.

As this study focused on TE among adults, subjects less than 15 years were excluded. The cases in which the cause of TE was not fully investigated were also excluded.

One hundred and twenty-one normal biopsies were included. Cases with a history of diarrhea, altered bowel habits, or abdominal pain were excluded even if the biopsy result was normal. Biopsies from subjects less than 15 years old were also excluded. The normal biopsies included were from subjects undergoing screening, cancer follow-up, or polyps—where the normal mucosa was also biopsied. Colonic biopsies from patients with vague abdominal pain who also underwent a gastric biopsy, showing histologically documented gastritis that explained the pain, were included.

The hematoxylin and eosin slides for all cases were reviewed to confirm the initial diagnosis, count the number of eosinophils, and check for features of eosinophil activation, including eosinophilic cryptitis, crypt abscesses, and degranulation [Figure 1].

The eosinophilic count was recorded as the number of eosinophils per HPF using a 10X ocular lens and a 40X objective lens, resulting in 400-fold magnification with a field area of 0.24 mm^2 using an Olympus BX51 microscope. The number of eosinophils in the most densely populated HPF, as well as those of four other HPFs, were recorded.

Statistical analysis

The data were presented on a Microsoft Excel sheet, version 16.12. A two-tailed t-test was used to compare the

Table 1: Demographic features of the cases and control groups

	Normal controls	PEC	Drug-induced	UC	CD
Number of cases	121	22	12	76	61
Percentage of females	45%	50%	50%	40.8%	52%
Age range	16-82	15-87	19-67	16-86	15-83
Mean age	53.9	46.8	43.25	40.6	38.9
Median age	56	49.5	43	41	41
SD	17.2	20.0	18.18	16.0	15.4

PEC=Primary eosinophilic colitis, UC=Ulcerative colitis, CD=Crohn's disease SD=Standard deviation

means of variables and a *P* value of < 0.05 was considered as significant. Confidence intervals (CIs) were calculated at a 95% level. Pearson's correlation coefficient was used to measure correlations between variables, where appropriate.

The receiver operating characteristic curve (ROC) analysis was used to find the cutoff point with the best sensitivity and specificity. The area under curve (AUC) was divided into five categories, which were, 0.90–1 as excellent, 0.80–0.90 as good, 0.70–0.80 as fair, 0.60–0.70 as poor, and 0.50–0.60 as fail.[20]

Ethical considerations

The University of Jordan ethical committee and the JUH Institutional Review Board (IRB number 67/2017/4288) approved this study.

RESULTS

This cohort comprised of 121 normal controls and 171 TE cases, which included 22 PEC, 76 UC, 61 CD, and 12 drug-induced cases. Table 1 details the demographic features of the study population.

Comparing eosinophil density and activation between cases and controls

Table 2 details the ECs in TE cases and controls. Using both counting methods showed a statistically significant difference between EC between both groups; $P < 0.000$ in both methods.

The ROC curve analysis of both counting methods are shown in Figure 2 (highest count) and Figure 3 (mean of five fields). The AUC in both is within the fair category (0.71 and 0.79, respectively). Several cutoff points were chosen and their respective sensitivity and specificity calculated [Table 3]. Taking 20 as a cutoff gave 80% sensitivity and 60% specificity if the mean of five fields method was used, whereas the same point had 92% sensitivity but 39% specificity if the highest count was used. In this latter method, 30 eosinophils per HPF achieved 80% sensitivity and 65% specificity.

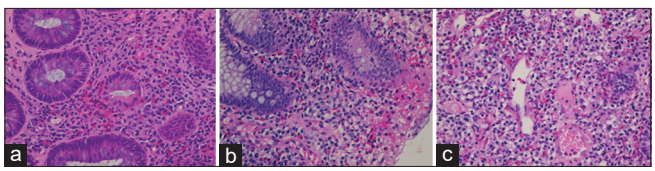

Figure 1: Tissue eosinophilia, 40X, (a and b) Eosinophils within lamina propria, (c) Eosinophilic cryptitis

Table 2: EC per HPF in TE cases and normal controls by using two counting methods: count in the HPF with the highest eosinophilic density compared to calculating the mean of five HPFs

	Normal controls	TE
HPF with the highest count		
Max	64	112
Mean	17.2	35.7
Median	13	32
SD	12.2	22.6
CI	2.2	3.4
Mean of five HPFs		
Max	39.8	66.6
Mean	11.7	24.2
Median	9	23.4
SD	9.0	22.6
CI	1.6	2.1

EC=Eosinophilic count, HPF=High-power field, TE=Colonic tissue eosinophilia, SD=Standard deviation, CI=Confidence interval calculated at 95%

Histologic features of eosinophil activation were rarely seen in the control group; eosinophilic cryptitis was noted in 9.1% compared with 55.6% of the TE cases. Degranulation and crypt abscesses were not seen in the controls.

Comparing eosinophil density and activation among the cases subgroups:

The PEC cases showed the highest number of eosinophils. The mean eosinophilic count in the HPF with the highest density was 55.3 compared with 46.8 in the drug-induced cases [Table 4].

Of the 61 CD cases, 32.8% were active and 67.2% quiescent. The mean of the maximum number of eosinophils in the active cases was 38.9 compared with

Table 3: Sensitivity and specificity of different cutoff points of ECs using the two studied counting methods

Cutoff point Eosinophils per HPF	Highest count		Mean of five HPFs	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
20	92	39	86	60
25	87	54	75	75
30	80	65	60	80
35	67	71	49	87
40	63	82	41	95
45	52	90	37	100
50	45	92	28	100

EC=Eosinophilic count, HPF=High-power field, Specificity=1- false positives

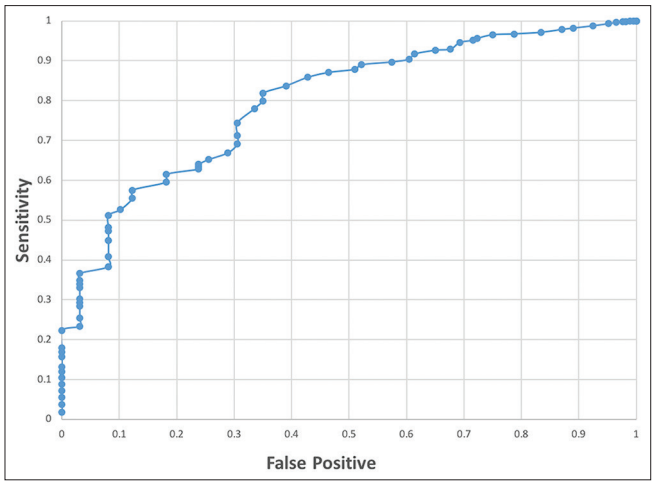

Figure 2: ROC curve, normal versus TE, using the highest count method. AUC = 0.71. sensitivity measures true positives. False-positive = 1- specificity

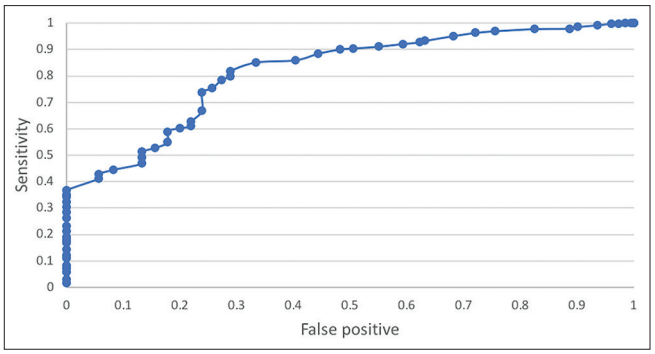

Figure 3: ROC curve, normal versus TE, using the mean of five HPFs. AUC = 0.79

18.6 in the quiescent cases (P = 0.0006). The mean number of eosinophils measured in five fields was also statistically significant (P = 0.0003). Seventy-six of the TE cases were because of UC, 57.9% were active and 42.1% quiescent. The maximum number of eosinophils in the active cases was 110 compared with 57 in the quiescent cases. There was a statistically significant difference between the mean of five fields (P = 0.0008) and maximum (P = 0.005) number of eosinophils per HPF between the two groups.

Table 5 summarizes features of eosinophilic stimulation and lymphoid aggregates. Degranulation was a universal feature in the study groups. There was a correlation between cryptitis and the mean of eosinophils per HPF with the highest density (Pearson's coefficient of 0.59). All the cases of PEC and drug-induced TE contained foci of cryptitis. There was no correlation between the eosinophil density and the rest of eosinophil activation features.

Comparing eosinophilic density and activation between primary and secondary TE

ECs were higher in the PEC compared with secondary TE. There was a statistical difference between the two groups if the highest count method was used. P = 0.001 but not if the mean of five fields considered P = 0.054.

Table 6 gives the P values comparing the number of eosinophils between each TE category and PEC. It shows that the most statistically significant difference is between PEC and both quiescent CD and UC. This applies to both counting methods.

There was no statistical difference between ECs (highest and mean) between PEC and drug-induced TE.

Table 4: Number of eosinophils per HPF in the TE groups as measured by the two counting methods

	PEC	Drug-induced	UC active	UC quiescent	CD active	CD quiescent	All secondary TE combined
Highest Count							
Mean	55.3	46.8	41.6	29.8	38.9	18.6	32
Median	49.5	43	35	28.5	32	15	36.3
SD	23.8	10.7	19.7	15.2	30.8	23.9	23.6
CI	10.0	6.05	5.8	5.3	13.5	6.3	3.6
Mean of Five Fields							
Median	25.7	30	29.1	21.4	21.7	10.4	25.6
Mean	32.4	30.8	31.4	21.6	24.3	12.2	26.7
SD	14.1	7.7	12.9	10.9	16.3	14.9	15.4
CI	5.89	4.4	3.8	3.8	7.1	3.3	2.3

HPF=High-power field, TE=Eosinophilic count, UC=Ulcerative colitis, CD=Crohn's disease, SD=Standard deviation, CI=Confidence interval

Table 5: Pearson's correlation coefficient between the mean of features of eosinophil activation and associated lymphoid aggregates in each group

	PEC number (%)	Drug number (%)	CD A number (%)	CD Q number (%)	UC A number (%)	UC Q number (%)	Pearson's coefficient
Degranulation	All	All	All	All	All	All	
Eosinophilic cryptitis	all	all	11 (55)	13 (13.7)	31 (70.5)	6 (18.8)	0.59
Eosinophilic abscesses	1 (4.6)	2 (16.7)	2 (10)	1 (2.4)	7 (15.9)	1 (3.1)	−0.12
Lymphoid aggregates	12 (54.5)	7 (58.3)	16 (80)	24 (58.5)	29 (65.9)	16 (50)	−0.21

PEC=Primary eosinophilic colitis, CD=Crohn's disease, A=Active, Q=Quiescent, UC=Ulcerative colitis

Table 6: *P* of ECs, comparing TE subgroups with PEC

Category	*P*, Highest count	*P*, Mean of five fields
CD A	0.104	0.003*
CD Q	0.000*	<0.000*
UC A	0.015*	0.776
UC Q	<0.000*	0.002*
Drugs	0.249	0.712

*Significant, EC=Eosinophil count, CD=Crohn's disease, A=Active, Q=Quiescent, UC=Ulcerative colitis

The ROC curves for both the highest [Figure 4] and mean [Figure 5] ECs showed an overlap between ECs in PEC and secondary TE. The AUC of both was in the fail category (0.56 for the highest count and 0.55 for the mean of five HPFs). This precludes finding a useful cutoff point to differentiate primary from secondary TE. For example, considering the highest count method, a cutoff point of 20 and 30 gave 100% sensitivity but 10% and 30% specificity, respectively.

Eosinophil activation was noted in all groups, and degranulation was present in all cases of primary and secondary TE. Eosinophilic cryptitis was a common feature in PEC, drug-induced TE, and active IBD.

DISCUSSION

This cohort of 171 TE cases and 121 normal controls shows that better sensitivity and specificity of detecting TE is obtained using a cutoff point of 30 eosinophils per HPF if counting the most densely populated field, and 20 if calculating a mean of five fields, combining these points with the presence of features of eosinophil activation improve diagnosing TE. It also shows that calculating the mean of five fields has no significant advantage over counting the most densely populated HPF. The study demonstrates that there is no reliable cutoff point to differentiate primary from secondary TE, and features of eosinophil activation cannot be relied on for this matter.

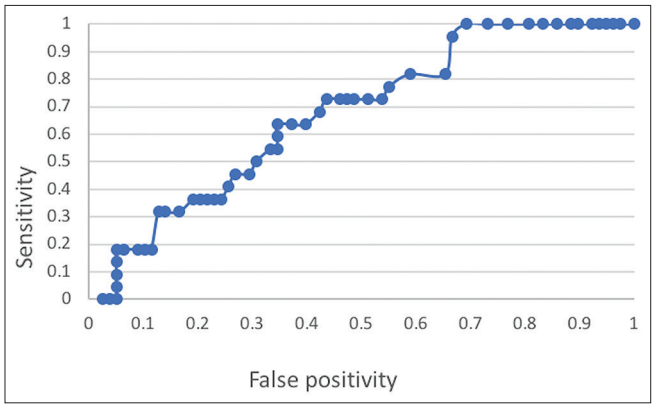

Figure 4: ROC curve, PEC versus secondary TE, using the highest count method. AUC = 0.56

In normal controls, the mean number of eosinophils per HPF in the most densely populated field is 17.2 and the mean + 1 SD is 29, this is close to our suggested 30 cutoff point as it gives 80% sensitivity and 65% specificity. If we were to use the mean + 2 SD (41 eosinophils), this would decrease the sensitivity to 63%, resulting in missing 37% of the cases. Although this point would increase specificity to 82%, in practical terms, increasing the detection rate is more important than decreasing false positives. However, pathologists and gastroenterologists need to recognize the overlap between normal and abnormal counts and evaluate cases on individual basis taking into consideration the presence of symptoms and the full clinical scenario to not overtreat TE. It is possible that the term "asymptomatic PEC" used by some researchers falls into this group of false positives. Indeed, most researchers are reluctant to diagnose PEC in asymptomatic patients,[12] which seems a valid approach.

The current practice of considering 20 as a cutoff point in the most densely populated field achieves 92% sensitivity but decreases specificity to 39%. This point is also too close to the mean of normal eosinophils (17.2), as such, we recommend increasing the cutoff point to 30. The previously suggested use of the highest count[17] or a multiple of the highest count[2] ignores the overlap between normal and TE cases, which will result in a high false-negative rate.

The mean of 17.2 eosinophils found in this study is close to that reported by DeBrosse[8] (15 per HPF) and Lowichik[9] (17 per HPF), but higher than that reported by, Lee (7 per HPF) and[13] Matsushita (9.4 in proximal colon and 2.0 in distal colon). This variability could be explained by geographic distribution[21] and the segments of the colon examined. Several studies documented higher counts in the right colon compared with the left colon.[10,12,22]

Our results indicate that counting eosinophils in the most densely populated field is sufficient; however, if a mean of

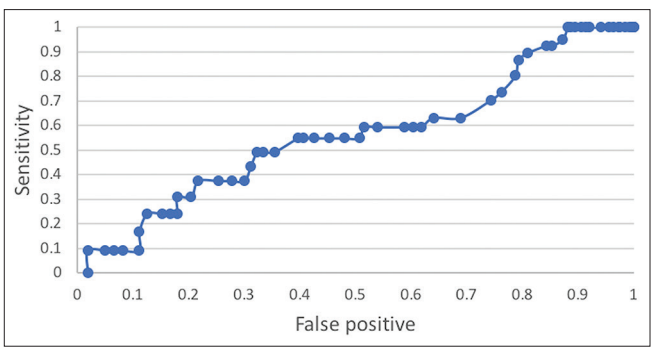

Figure 5: ROC curve, PEC versus secondary TE, using the mean of five HPFs method AUC = 0.55

five fields is taken into consideration, the overlap between normal and TE will improve slightly (as judged by AUC, 0.71 for the highest count compared with 0.79 for the mean of five HPFs). If this method is used, we suggest using 20 eosinophils per HPF as the cutoff point, this will achieve 80% sensitivity and 60% specificity. Again, this point is close to the mean + 1 SD (29.7) if this method is used.

Although the ROC curve and AUC suggest that taking the mean of five fields is slightly better than taking the highest count, the points we are suggesting obtain the same sensitivity (80%). In terms of specificity, using the highest count method is better as it achieves 65% specificity compared with the 60% achieved by counting five fields. Counting one field is also more practical and less time-consuming.

Features of eosinophil activation are useful clues to differentiate TE from normal as they are common features of TE cases, but they are rarely seen in normal controls. These results are similar to those reported by Turner *et al*.[12]

Although PEC as a group has higher ECs than secondary TE, our results show too much overlap between ECs in these two entities (AUC close to 0.5). This applies to the two counting methods and precludes finding a meaningful cutoff point to differentiate them. Secondary TE contains a heterogeneous group of diseases, some of which have ECs as high as those in PEC, this is particularly obvious in cases of drug-induced TE. Active IBD, both Crohn's and UC types, show significantly higher ECs than quiescent cases, and UC exhibits higher ECs than CD in both the active and quiescent phases. Features of eosinophil activation are also not helpful in differentiating PEC from secondary TE as revealed by the insignificant Pearson's correlation coefficient. Separating primary from secondary causes of TE remains a clinicopathological question that needs full clinical review.

Limitations

The previously reported geographical differences of normal ECs[21] limit our results to our geographical area and cannot be confidently generalized. However, the normal counts in our sample are close to those reported from other geographical areas, including the United States of America (USA), although they differ from results from Japan. More research is needed to verify the significance of these geographical differences.

Another limitation of this study is that we did not evaluate eosinophils in the right and left colonic biopsies separately because in a large percentage of cases the exact biopsy site was not specified. More studies investigating normal numbers of eosinophils in the right and left colon are needed and can lead to separate cutoff points for each segment of the colon. However, our study reflects the usual daily practice of submitting biopsies in one container labeled as random colonic biopsies.

CONCLUSION

We recommend using 30 eosinophils per HPF as a cutoff point to diagnose TE and count only the most densely populated field. In view of the lack of distinguishing histological features, clinicopathological correlation is essential to separate PEC from secondary TE.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Conner JR, Kirsch R. The pathology and causes of tissue eosinophilia in the gastrointestinal tract. Histopathology 2017;71:177-99.
2. Collins MH, Capocelli K, Yang GY. Eosinophilic gastrointestinal disorders pathology. Front Med 2017;4:261.
3. Samiullah, Bhurgri H, Sohail U. Eosinophilic disorders of the gastrointestinal tract. Prim Care 2016;43:495-504.
4. Alfadda AA, Storr MA, Shaffer EA. Eosinophilic colitis: An update on pathophysiology and treatment. Br Med Bull 2011;100:59-72.
5. Silva J, Canao P, Espinheira MC, Trindade E, Carneiro F, Dias JA. Eosinophils in the gastrointestinal tract: How much is normal? Virchows Arch 2018;473:313-20.
6. Egan M, Furuta GT. Eosinophilic gastrointestinal diseases beyond eosinophilic esophagitis. Ann Allergy Asthma Immunol 2018;121:162-7.
7. Alnaser S, Aljebreen A. Endoscopic ultrasound and hisopathologic correlates in eosinophilic gastroenteritis. Saudi J Gastroenterol 2007;13:91-4.
8. DeBrosse CW, Case JW, Putnam PE, Collins MH, Rothenberg ME. Quantity and distribution of eosinophils in the gastrointestinal tract of children. Pediatr Dev Pathol 2006;9:210-8.
9. Lowichik A, Weinberg AG. A quantitative evaluation of mucosal eosinophils in the pediatric gastrointestinal tract. Mod Pathol 1996;9:110-4.
10. Saad AG. Normal quantity and distribution of mast cells and eosinophils in the pediatric colon. Pediatr Dev Pathol 2011;14:294-300.
11. Chernetsova E, Sullivan K, de Nanassy J, Barkey J, Mack D, Nasr A, *et al*. Histologic analysis of eosinophils and mast cells of the gastrointestinal tract in healthy Canadian children. Hum Pathol 2016;54:55-63.
12. Turner KO, Sinkre RA, Neumann WL, Genta RM. Primary colonic eosinophilia and eosinophilic colitis in adults. Am J Surg Pathol 2017;41:225-33.
13. Matsushita T, Maruyama R, Ishikawa N, Harada Y, Araki A, Chen D, *et al*. The number and distribution of eosinophils in the adult human gastrointestinal tract: A study and comparison of racial and environmental factors. Am J Surg Pathol 2015;39:521-7.
14. Kiss Z, Tel B, Farkas N, Garami A, Vincze A, Bajor J, *et al*. Eosinophil counts in the small intestine and colon of children without apparent gastrointestinal disease: A meta-analysis. J Pediatr Gastroenterol Nutr

2018;67:6-12.
15. Lee EH, Yang HR, Lee HS. Quantitative analysis of distribution of the gastrointestinal tract eosinophils in childhood functional abdominal pain disorders. J Neurogastroenterol Motil 2018;24:614-27.
16. Gaertner WB, Macdonald JE, Kwaan MR, Shepela C, Madoff R, Jessurun J, *et al*. Eosinophilic colitis: University of Minnesota experience and literature review. Gastroenterol Res Pract 2011;2011:857508.
17. Alhmoud T, Hanson JA, Parasher G. Eosinophilic gastroenteritis: An underdiagnosed condition. Dig Dis Sci 2016;61:2585-92.
18. Talley NJ, Shorter RG, Phillips SF, Zinsmeister AR. Eosinophilic gastroenteritis: A clinicopathological study of patients with disease of the mucosa, muscle layer, and subserosal tissues. Gut 1990;31:54-8.
19. Behjati S, Zilbauer M, Heuschkel R, Phillips A, Salvestrini C, Torrente F, *et al*. Defining eosinophilic colitis in children: Insights from a retrospective case series. J Pediatr Gastroenterol Nutr 2009;49:208-15.
20. Safari S, Baratloo A, Elfil M, Negida A. Evidence based emergency medicine; Part 5 receiver operating curve and area under the curve. Emergency (Tehran, Iran) 2016;4:111-3.
21. Pascal RR, Gramlich TL, Parker KM, Gansler TS. Geographic variations in eosinophil concentration in normal colonic mucosa. Mod Pathol 1997;10:363-5.
22. Polydorides AD, Banner BF, Hannaway PJ, Yantiss RK. Evaluation of site-specific and seasonal variation in colonic mucosal eosinophils. Hum Pathol 2008;39:832-6.