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ORIGINAL ARTICLE

Seasonal variation of eosinophil counts in histologically normal colonic mucosal biopsies

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Abstract

Introduction: The eosinophil counts in colonic biopsies are affected by geographical and possibly seasonal variations. This study aims to investigate the significance of seasonal variations of eosinophil counts in histologically normal colonic mucosal biopsies. *Materials and Methods:* This is a retrospective, cross sectional study that included 337 cases of normal colonic biopsies. The number of eosinophils per high power field was counted in the most densely populated area. The eosinophilic counts were compared among genders, age groups, biopsy sites and in various months and seasons. Two tailed T-test was used to compare means and a p value < 0.05 was considered significant. *Results:* 173 (51%) of cases were from males. The age range was between 18-82 with the mean being 51.7 years (SD= 17.5). 181 (54%) biopsies were from the right colon and 156 (46%) from the left colon. There was a statistically significant difference between eosinophil counts in the right colon (mean 20.2, SD 13.2) and left colon (mean 13.8, SD10.1); p value <0.001. The mean eosinophil counts was highest in autumn (21.1) followed by spring (18.3). The counts in winter and summer were close (15.2 and 15.1 respectively). There was a statistically significant difference between counts in autumn and summer (p=0.013) and between autumn and winter (p=0.008). However, there was no statistically significant differences between autumn and spring counts (p=0.183). When stratified according to site, this pattern of statistical significance was observed in the right colon but not the left colonic mucosal biopsies. *Conclusion:* There are significant seasonal variations of eosinophil counts in normal colonic biopsies which are more pronounced in the right colon. Pathologists and gastroenterologists need to be aware of these variations and to take them into account when determining if a patient has tissue eosinophilia.

Keywords: Eosinophils, large intestine, Jordan, colitis, seasonal variation

INTRODUCTION

Eosinophils are bone marrow-derived granulocytes that differentiate in the bone marrow and then distributed in the blood, lungs, uterus, fatty tissues, spleen and the gastrointestinal tract (GIT).[1,2] T-helper 2 cytokines, mainly IL-5, play an important role in eosinophil differentiation and survival.[2] Eosinophilic granules contain cationic proteins that include major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPO).[1]

Eosinophils are part of the innate immune system that are stimulated in response to allergic reactions and parasitic infections.[1,3] However, they are also believed to play an important role in the adaptive immunity through regulating T and B cell functions. They promote plasma cell survival and keep a balance between T-helper and T-regulatory cells in the gastrointestinal tract (GIT) and in the lungs.[4,5] After exposure to allergens, eosinophils act as antigen presenting cells; they express MHC class II and costimulatory molecules.[2,4]

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Although the gastrointestinal tract contains the largest reservoir of eosinophils, their function in the GIT mucosa is not fully understood. Eosinophils are thought to increase IgA-producing plasma cells, promote IgA class-switching, increase mucus secretions, and induce Peyer's patches development.[2, 5-7]

Increased eosinophils beyond normal counts in the GIT cause inflammatory disorders called eosinophilic gastrointestinal disorders (EGID). The accumulation of eosinophils can occur in any segment of the GIT hence EGID is a group of disorders that includes eosinophilic esophagitis, eosinophilic gastritis, eosinophilic enteritis and eosinophilic colitis (EC).[8,9] Increased eosinophils in the colon can occur as part of the spectrum of EGID (Primary EC) or as a secondary reaction (secondary tissue eosinophilia) to several disorders including food allergy, inflammatory bowel disease, infections, drugs and neoplasms.[10-13]

To diagnose primary and secondary colonic tissue eosinophilia we need to define a reference cut-off point of the eosinophil counts in normal colonic mucosal biopsies, however there is no consensus in the published literature regarding such a cut-off point. In the clinical practice, many pathologists and gastroenterologists use 20 eosinophils/HPF as the upper limit of normal[14-16], others use 30 eosinophils /HPF[17], whereas some researchers suggested using 50 eosinophils/HPF[18], which is the maximum number reported in normal biopsies**.** Collins *et al*. recommended using twice the maximum of normal counts as the accepted higher limit of normal which, in their study, was 100/HPF in the cecum and ascending colon, 84/HPF in the transverse and descending colon and 64/HPF in the recto-sigmoid.[11] A study from our institution (Jordan University Hospital (JUH)) suggested using 30 eosinophils per HPF as this achieved 80% sensitivity and 65% specificity.[19] The variation in the suggested cut-off points is partly explained by the geographical variation in eosinophil counts and by the differences in counts among children and adults. Seasonal variations can also be a cause of the variability of cut-off points reported in the literature. Polydorides studied seasonal variations in eosinophil counts and reported higher counts in April and May, corresponding to periods of highest pollen counts, but that was not statistically significant in his cohort.[20] No other studies addressed this issue in colonic biopsies, however, Lwin found no significant seasonal variation of eosinophil counts in normal gastric biopsies.[21] Janson *et al*. reported a significant seasonal variation in the eosinophil cationic protein in the serum of atopic patients ($P < 0.05$).[22]

This study aims to investigate the seasonal variation of eosinophil counts in histologically normal colonic biopsies. If a significant variation is documented, this needs to be taken into consideration when colonic tissue eosinophilia is suspected histologically.

MATERIALS AND METHODS

This is a retrospective, cross-sectional study conducted at JUH between 1st January 2016 to 31st December 2019. A total of 337 cases of histologically normal colonic biopsies were included. The study was approved by the University of Jordan Research Deanship and by the Institutional Review Board (IRB) at JUH (IRB Number: 8285/10/2021). No funds were received for this project.

The computerised histopathology reports at the histopathology department at JUH were searched for normal colonic biopsies. The reports were reviewed with special attention to the clinical data available and the reason to perform the biopsy. Inclusion criteria were: histologically normal colonic biopsies taken from adult patients (18 years and older) undergoing the colonoscopy for any of the following reasons: *i:* screening, *ii:* follow up after cancer (only biopsies away from the anastomotic site were included), *iii:* having polyps (where normal mucosa away from the polyp was biopsied), *iv:* vague abdominal pain explained by gastric related causes including H. pylori infection. Biopsies from children below 18 were excluded as the aim of the study was to study eosinophil numbers among the adult population. Cases where there is history of diarrhoea, changes in bowel habits or bleeding per rectum were excluded as these are the most common symptoms of eosinophilic colitis. Cases where the exact site of the specimen (right vs left colon) was not identified were also excluded. The final number of included cases was 337 cases.

For the included cases demographic features were recorded including age, gender, site of the biopsy and the month the specimen was taken.

Eosinophils were counted in the biopsies from the cecum, ascending colon, transverse colon, descending colon, sigmoid and rectum. Biopsies from the cecum, ascending and transverse colon were regarded as right sided whereas biopsies from the descending colon, sigmoid and rectum

were regarded as left sided. Some biopsies were received in the lab labelled as right or left colon without further specification of site.

To investigate for seasonal variations, December- February were classified as winter, March- May as spring, June-August as summer and September- November as autumn.

The archived Haematoxylin and Eosin slides were retrieved, and the biopsies were reviewed to confirm that the biopsies were histologically normal and to count the number of eosinophils in each case. Eosinophils were counted in the high-power field with the highest density of eosinophils. A published study from our institution proved that this method is superior to taking an average of 5 HPFs[19], and in histopathology practice the field with the highest eosinophil count is usually considered. Eosinophil count was performed using Olympus BX 53 microscope, with a high-power field diameter of 0.55mm, and field area of 0.238mm^2. All cells with eosinophilic granules were counted even if the nucleus was inconspicuous. For cases where more than 20 eosinophils per HPF were found, the computerised clinical records were reviewed to confirm that these patients didn't have a possible reason explaining the high counts.

The data was presented on Microsoft Excel sheet, version 16.12. Categorical data was presented as numbers and percentages. The mean, median and standard deviation (SD) were calculated for continuous data. Two tailed T-test was used to compare the means and a significant p value was considered to be < 0.05. Confidence intervals were calculated at 95% level.

RESULTS

A total of 337 histologically normal colonic biopsies were included in this study. 173 (51%) of which were from male patients and 164 (49%) from female patients; male to female ratio was 1.05:1. The age range was between 18-82 years with the mean age being 51.7 years (SD= 17.5). 181 (54%) biopsies were from the right colon and 156 (46%) were from the left colon. The number of eosinophils per HPF revealed high variation and was between 1-75 eosinophil per HPF (Figure 1).

There was no statistically significant difference between eosinophil counts in males and females (p= 0.62) with the mean number of eosinophils/HPF being 17.0 (SD 11.9) among males and 17.6 (SD 12.7) among females.

The mean count of eosinophils per HPF showed some variation among different age groups. Table 1 shows the mean counts of eosinophils according to age. Comparing the mean number of eosinophils in different age groups showed two peaks, one in patients below 20 (mean= 22.1), but this was statistically

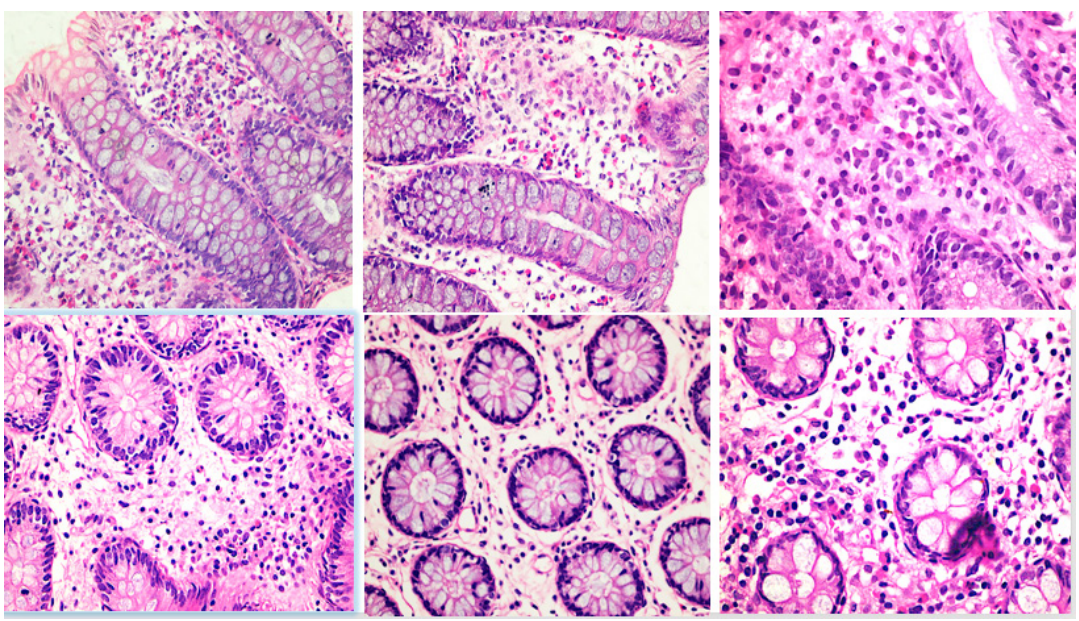

FIG. 1: Eosinophil Counts in various segments of the large intestine, clockwise: cecum, ascending colon, transverse colon, descending colon, sigmoid and rectum. In these samples the eosinophil count decreases from the cecum to the rectum. H&E Stain 400X.

TABLE 1: Eosinophil counts among different age groups

Age group	Number of cases	Eosinophil Count (per HPF)
<21	12	22.1
(21-30)	47	8.9
(31-40)	27	19.9
(41-50)	66	13.9
(51-60)	69	18.8
(61-70)	51	14.5
(71-80)	60	19.0
>80	5	30.0

(P = 0.17 below 21 years, P = 0.02 above 80 years)

insignificant (p=0.17). The other peak was in those above 80 (mean= 30) and this was statistically significant (P= 0.02), however this result should be interpreted cautiously as there were only 5 cases above 80 years of age in our study.

The studied cases included 181 cases from the right colon and 156 from the left colon. There was a statistically significant difference between eosinophil counts in the right (mean 20.28, SD 13.2) and left colon (mean 13.8, SD10.1); p value was <0.001. Table 2 details eosinophil counts in the right and left colon as well as in the various anatomical regions. Notably there is no statistical difference between eosinophil counts among the various regions of the right colon but there is a

TABLE 2: Comparing eosinophil counts among anatomical regions of the large intestine

	Eosinophil count	Mean (CI)	median	SD	P value
Right (all)	181	20.3 (18.4-22.2)	17	13.2	P value (compared against caecum)
Cecum	22	20.7 (14.2-27.5)	18	14.7	-
Ascending	4	19.3 (2.5-36.1)	18	10.6	0.85
Transverse	25	21.0 (15.4-26.6)	20	13.6	0.94
Right NOS	130	20.1 (17.8-22.4)	16	13.1	0.84
Left (All)	156	13.9 (12.3-15.5)	11	10.2	P value (compared against rectum)
Descending	7	22.1 (12.4-31.8)	19	10.5	<0.001*
Sigmoid	30	13.0 (9.6-16.4)	11	9.1	0.03*
Rectum	31	8.4 (5.2-12.6)	7	6.5	-
Left NOS	88	15.3 (12.6-18)	13	10.7	0.001*

CI - confidence interval, SD - standard deviation, NOS - not otherwise specified. *p value of <0.05 is considered as significant.

TABLE 3: Seasonal variation of eosinophil counts

	Number of cases	Mean Eosinophils /HPF	Median Eosinophils/ HPF	SD	P value with Spring	P value with Autumn
Spring	125	18.3	15	12.08	-	0.183
Summer	58	15.1	13	10.29	0.084	0.013*
Autumn	52	21.1	18	14.47	0.184	-
Winter	102	15.2	11.5	11.98	0.057	0.008*

*p value of <0.05 is considered as significant.

significant difference between the counts in the rectum, which had the lowest counts, and the rest of the left colon regions.

Regarding seasonal variation, our results showed differences in the eosinophil counts among different months and seasons. The mean eosinophil counts were highest in autumn (21.1) followed by spring (18.3). The counts in winter and summer were close (15.2 and 15.1 respectively). There was a statistically significant difference between autumn counts and summer counts (p=0.013) and between autumn counts and winter ones (p=0.008). However, there was no statistically significant differences between autumn and spring counts (p=0.183). Notably there was no statistically significant difference between spring eosinophil counts and the other seasons (Table 3).

Eosinophil counts also varied among the months of the year. The highest counts were in September and October (23/HPF each) followed by April (21/HPF). The least count was in July (12/HPF) (Figure 2).

The seasonal variability of eosinophil counts applied to the left and right colon as well. The mean counts were highest in autumn in both right and left colonic biopsies. However, the difference between autumn counts in one hand and summer and winter in the other was statistically significant in the right colonic biopsies only (p values 0.031 and 0.009 respectively). Again, there was no significant difference between autumn and spring

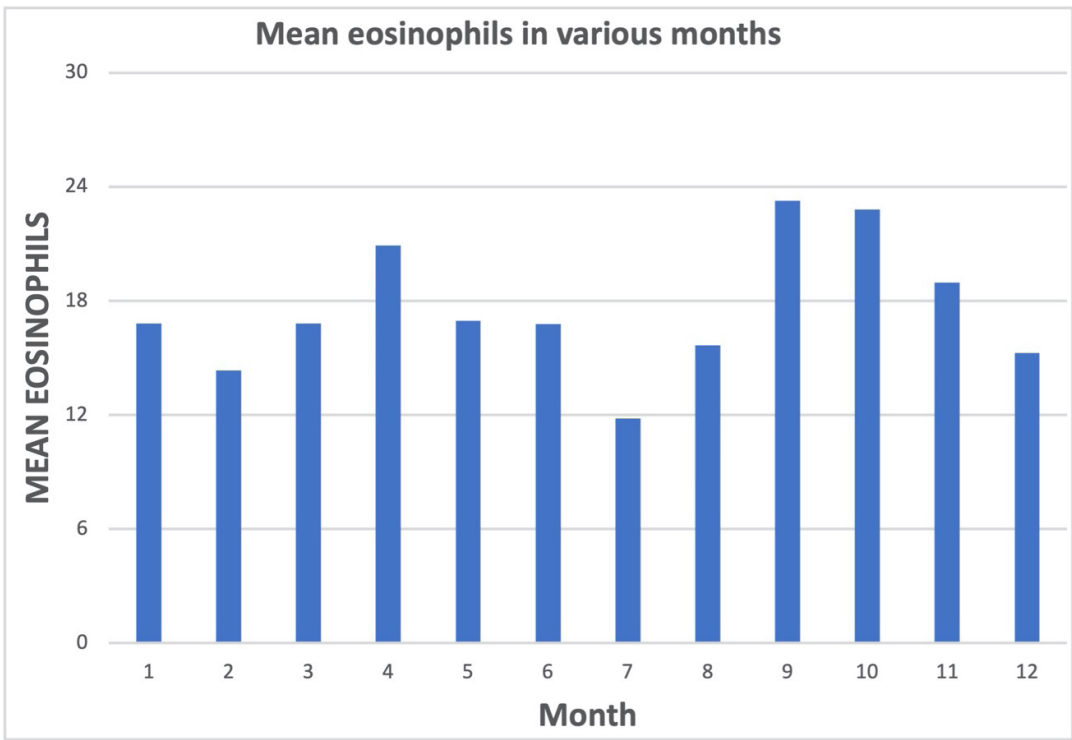

FIG. 2: Eosinophil counts differ among months of the year with the highest counts being in September and October.

counts in both right and left colonic biopsies, (Table 4).

DISCUSSION

This is a retrospective cross-sectional study which included 337 histologically normal large bowel mucosal biopsies. It contains the largest cohort of histologically normal colonic cases in the published literature and is the first from our region to describe the seasonal variations of eosinophils in normal colonic biopsies. The issue of seasonal variation of eosinophils in the colon is not sufficiently addressed in literature. Polydorides *et al*. were probably the first to study seasonal variations in eosinophil counts and they reported higher counts in April and May, which are the periods of highest pollen counts, however they didn't document a statistically significant difference.[20] No other studies addressed this issue in colonic biopsies, but some studies looked into eosinophil counts in the esophagus and stomach. Lwin found no significant seasonal variation of eosinophil counts in normal gastric biopsies.[21] Janson *et al*. reported a significant seasonal variation in eosinophil cationic protein serum levels in the atopic pollen-positive subjects ($P < 0.05$).[22] Other studies reported a mild seasonal variation in the diagnosis of esophageal eosinophilia, with cases more frequently diagnosed during summer months.[23] Whereas others reported diagnosing larger number of eosinophilic esophagitis (EE) cases in spring.[24] Some authors reported increased diagnosis of eosinophilic esophagitis in summer and spring.[25] Interestingly, a recent article has described seasonal variation in intraepithelial eosinophils as well as lymphocytes in esophegeal biopsies being lowest during autumn and highest during spring.[26] This seasonal variation was challenged by a study that found close numbers of EE cases diagnosed in all 12 months and during the four seasons.[27]

This discrepancy in literature calls for more investigation of the subject especially in the colon where there is scarcity of information regarding seasonal effect on eosinophil counts in colonic biopsies. Our results show that eosinophil counts in winter and summer were close (15.2 and 15.1 respectively) but was higher in spring (18.3) however there was no statistically significant difference between spring eosinophil counts and the other seasons. The highest mean of eosinophil counts was in autumn (21.1) and this was statistically significant from summer and winter counts (p= 0.013 and 0.008 respectively) but not spring ones (p=0.183). The seasonal variability of eosinophil counts applies to the left and right colon as well. The mean counts were highest in autumn in both right and left colonic biopsies. However, the difference between autumn counts in one hand and summer and winter in the other was statistically significant in the right colonic biopsies only (p values 0.031

TABLE 4: Seasonal variation of eosinophil counts in the right and left colonic mucosal biopsies

RIGHT COLON	Number of cases	Mean	Median	SD	P value in comparison with Autumn	P value in comparison with Spring
Autumn	28	25.6	25	15.6	-	0.223
Spring	64	21.8	19	13.1	0.223	-
Summer	31	17.8	16	11.3	0.031*	0.157
Winter	58	17.4	13.5	12.4	0.009*	0.062
LEFT COLON						
Autumn	24	15.9	14.5	11.2		0.624
Spring	61	14.7	13	9.8	0.624	
Summer	27	12	12	8.1	0.156	0.212
Winter	44	12.4	10	11.0	0.21	0.257

*p value of <0.05 is considered as significant.

and 0.009 respectively). There was no significant difference between autumn and spring counts in both right and left colonic biopsies.

Our results differ from those reported in literature in respect to the season of highest count. In our study the highest counts were in autumn followed by spring. The spring peak can be explained by the high pollen counts in spring but the autumn peak is more difficult to explain. However, certain types of pollens do peak in autumn[28,29] but unfortunately there are no studies about pollen distribution in Jordan.

The relation between pollen seasons and gastrointestinal diseases is not well understood but one well known example is pollen food allergy, also known as oral allergy syndrome, which is an IgE mediated allergy to certain types of foods especially fruit and vegetables. This type of allergy results from a cross reaction between food epitopes and certain pollens. The disease gets worse during the high pollen seasons.[30]

Although some studies show increased diagnosis of eosinophilic esophagitis in pollen seasons, there is no evidence that inhaled allergens like pollens are related to the pathogenesis of this disease.[25] A relation of pollen antigens and colonic eosinophilia will be even more difficult to comprehend. However, cross reactivity with certain types of food can play a role.

We investigated the seasonal variation of eosinophil counts in right and left colonic biopsy. Curiously, the variation was statistically significant in the right colon but not the left. Eosinophil counts were documented in previous literature to be higher in the right colon than in the left and our results are in line with literature in this aspect. Our results show a significant difference of normal counts between the right and the left colon ($p < 0.001$) with a decreasing number of eosinophils as we move from the cecum to the rectum. This trend is similar to that reported in previous studies.[15,24,31] Our results also showed no significant difference of eosinophil counts between males and females ($p = 0.62$). Comparing the mean number of eosinophils in different age groups shows two peaks, one in patients below 20 (mean= 22.1), but this was not statistically significant ($p=0.17$). The other peak was in those above 80 (mean= 30) and this was statistically significant ($P = 0.02$), however this needs to be interpreted cautiously as there were only 5 cases above 80 years of age.

Limitations

This is a single institution study that included a retrospective cohort of cases. In some cases, the exact site of the biopsy was not recorded (received labelled as right or left without further qualification). Because of the geographic variation of eosinophil counts, our results cannot be generalised and are probably relevant only to our population.

CONCLUSION

There are significant seasonal variations of eosinophil counts in histologically normal colonic mucosal biopsies which are more pronounced in the right colonic biopsies. Pathologists and gastroenterologists need to be aware of these variations and to take them into account when determining if a patient has tissue eosinophilia. A common scenario in clinical practice is to be faced with a normal biopsy with some increase in eosinophil counts. Determining if this increase is a variant of normal is an important decision pathologists need to make. This decision has to take into account the site of the biopsy, patient's history, endoscopic findings, geographic area as well as the seasonal variations.

Conflict of interests: The authors declare no conflicts of interest.

Authors' contribution: Conceptualisation: HA. Methodology: HA, SS, JO. Formal analysis: SS. Funding acquisition (no funding) Project administration: JO, MA, LA, OA. Visualisation: JO, MA, AB, LA. Writing-original draft: HA, JO, SS. Writing-review and editing: LA, SS, AB, MA, OA. Approval of final manuscript: all authors.

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