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Stereological evaluation of ganglion cells in the sigmoid colon in primary and recurrent sigmoid volvulus

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Aim: To clarify the role of ganglion cells in Auerbach's plexus of the sigmoid colon in the development and recurrence of sigmoid volvulus (SV).

Materials and methods: Pathological block samples of colonic resection materials of 28 patients who underwent sigmoidectomy for SV and, for comparison, samples of 28 patients who underwent sigmoidectomy or low anterior resection for rectosigmoid cancer (RSC) were analyzed using stereological methods.

Results: The mean numerical density of ganglion cells was significantly lower in the SV group than in the RSC group (140.70 mm^{-2} and 669.42 mm^{-2}, respectively, $P = 0.005$), whereas there was no significant difference between the revolvulus and nonrevolvulus SV groups (128.45 mm^{-2} and 185.67 mm^{-2}, respectively, $P > 0.05$). Similarly, the mean numerical density of ganglion cells in elderly SV patients was found to be significantly lower than that in nonelderly SV patients (66.57 mm^{-2} and 214.86 mm^{-2}, respectively, $P = 0.002$), while there was no significant difference between elderly and nonelderly RSC patients (638.60 mm^{-2} and 705.00 mm^{-2}, respectively, $P > 0.05$).

Conclusion: SV may be related to a decreased numerical density of ganglion cells in Auerbach's plexus, and the degenerative loss of ganglion cells due to advancing age is possible in elderly SV patients.

Key words: Sigmoid colon, volvulus, ganglion cell, Auerbach's plexus

1. Introduction

Sigmoid volvulus (SV), known as far back as ancient Egypt, recognized by Hippocrates (1,2) and first described by von Rokitansky in 1836 (3), is a rarely observed type of large bowel obstruction in which the sigmoid colon wraps around itself and its own mesentery (4–6). Some predisposing factors for the development and recurrence of SV have been suggested and documented, including the presence of a redundant sigmoid colon with a high and narrow mesentery, advanced age, male sex, pregnancy, high altitudes, a high-fiber vegetable diet, habitual constipation, and diseases such as adhesions, omphalomesenteric abnormalities, malrotations, intussusceptions, appendicitis, carcinomas, and particularly congenital or acquired megacolon (1–7). However, the role of the numerical density of the ganglion cells in the neural plexus has not been well investigated (8). This study was designed to investigate the relationship between the numerical density of ganglion cells in Auerbach's plexus of the sigmoid colon and SV, using a stereological method.

2. Materials and methods

In this study, pathological samples from 28 consecutive patients who underwent sigmoidectomy for SV and, for comparison, samples from 28 consecutive patients who underwent sigmoidectomy or low anterior resection without chemotherapy or radiotherapy for obstructive rectosigmoid cancer (RSC) in a 10-year period between January 2002 and January 2012 were analyzed. This study was approved by the Institutional Review Board/Ethics Committee. No patient with neuromuscular disease or with bowel ischemia/gangrene was included the study. All fresh samples were obtained in the Department of General Surgery of the Faculty of Medicine of Atatürk University from the middle part of the resected sigmoid colon in the

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SV group and from nontumoral tissue in the RSC group. Fresh resection materials were embedded in paraffin after tissue processing steps with a tissue-processing device (Leica TP1050) in the Department of Pathology. These paraffin blocks were cut into 5-µm sections, and every 21st section was selected according to the systematic random sampling rules used in modern cytology (9–11). Approximately 15–20 sections from each block were stained with hematoxylin-eosin (H&E) for stereological examination. Auerbach's plexus, the area between the circular and longitudinal smooth muscle borders, was identified and marked using Stereo Investigator 9.0 software at low magnification (Figure 1) with a microscope (Leica 4000B). The number and the density of the frames were selected in this marked area using the fractionator component of the Stereo Investigator system. An appropriate counting frame size was then determined by the Stereo Investigator automatically (Figure 2), and ganglion cells were counted using a fractionator probe at high magnification (Figure 3).

In the first step, the numerical density of ganglion cells in Auerbach's plexus was estimated for the every patient in the SV and RSC groups using the following formula:

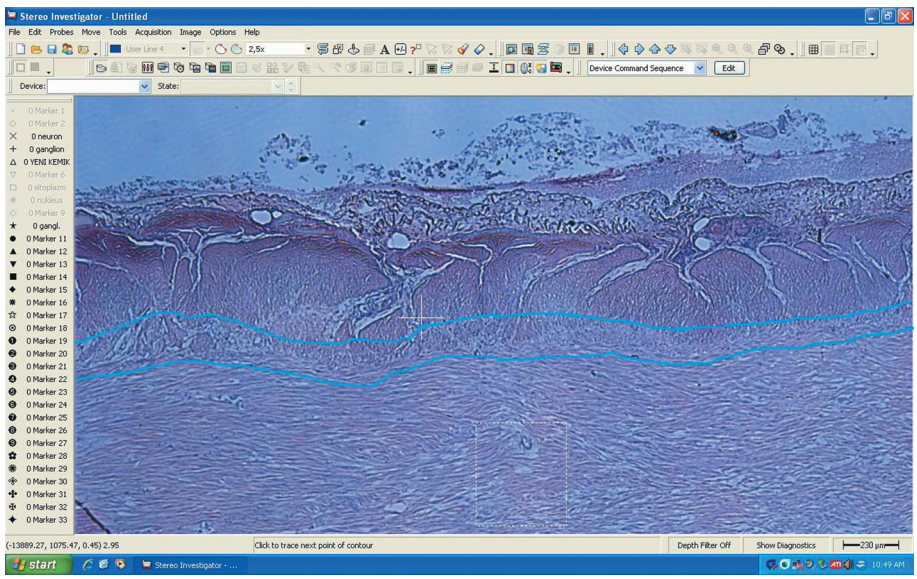

Figure 1. Marked Auerbach's plexus between the longitudinal and circular muscle tunics at 2.5× magnification. Section thickness: 5 µm, staining: H&E.

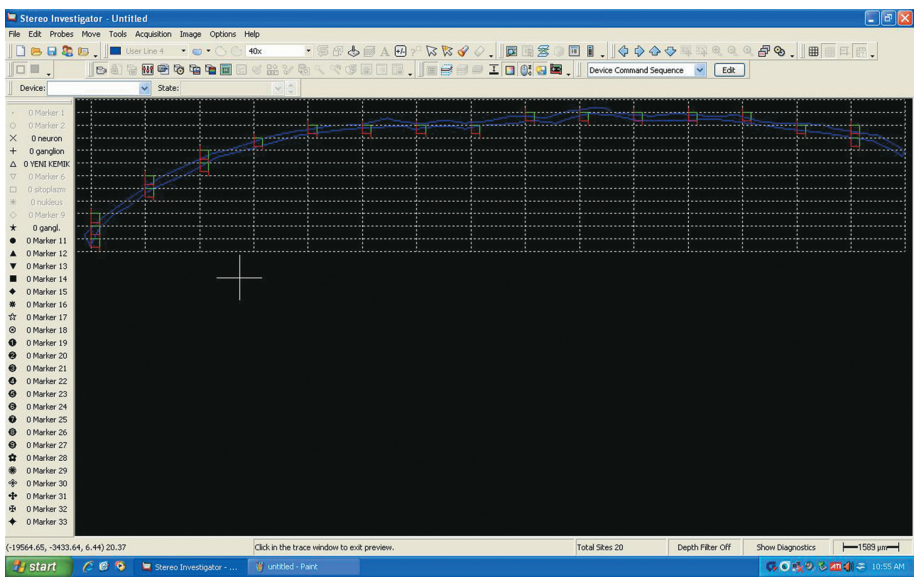

Figure 2. Determination of the number of sampling sites and the frame size for counting.

$$N_v = \frac{\Sigma Q}{\Sigma S \times A}$$

where N_v is numerical density, ΣQ is the total markers counted, ΣS is the number of sampling sites, and A is the counting frame area in μm². In the second step, we divided the SV patients into recurrent (revolvulus, recurrence after endoscopic or surgical detorsion, without resection) and primary (nonrevolvulus) cases, and we compared their mean numerical densities of ganglion cells. In the third step, samples obtained from SV and RSC cases were divided into 2 subgroups according to the patient's age (60 years of age and older, and under 60 years old) to determine the role of advanced age on the numerical density of ganglion cells in Auerbach's plexus.

To evaluate the significance of differences between these 3 pairs of groups, we used the independent samples t-test (2-tailed, significance limit of P = 0.05) with SPSS 15.0 for Windows.

3. Results

There were 28 patients in the SV group and 28 patients in the RSC group (Table 1). The mean numerical densities of ganglion cells in Auerbach's plexus were 140.70 mm^{-2} and 669.42 mm^{-2} for the SV and RSC groups, respectively. Thus, the mean numerical density of ganglion cells was significantly lower in the SV group (P = 0.005) (Table 2). In contrast, there was no significant difference between the numerical densities of ganglion cells in the revolvulus (n = 6) and nonrevolvulus (n = 22) SV groups (128.45 mm^{-2} and 185.67 mm^{-2}, respectively, P > 0.05). When the mean numerical density of ganglion cells for the 14 SV patients who were 60 years of age or older was compared with that for the 14 SV patients who were under 60 years of age, the numerical density of ganglion cells was found to be significantly lower in the elderly group (66.57 mm^{-2} and 214.86 mm^{-2}, respectively, P = 0.002). Nevertheless, there was no significant difference between the numerical

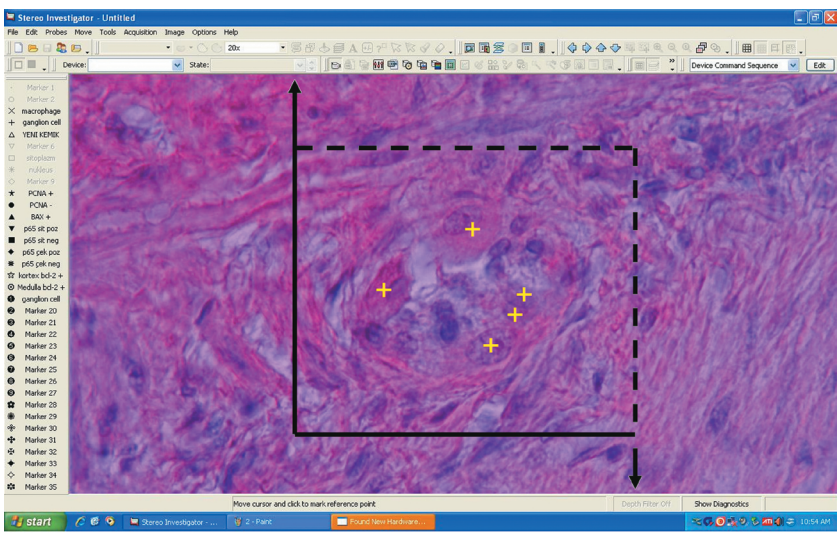

Figure 3. Unbiased counting frame application to a slice at 40× magnification. Section thickness: 5 μm, staining: H&E.

Table 1. Characteristics of the patients.

Characteristic	Sigmoid volvulus (n = 28)	Rectosigmoid cancer (n = 28)
Age, years		
Minimum	23	28
Maximum	81	84
Mean	58.6	62.2
Sex, n (%)		
Male	23 (82.1%)	15 (53.6%)
Female	5 (17.9%)	13 (46.4%)
Type of resection, n (%)		
Sigmoidectomy	28 (100.0%)	4 (14.3%)
Low anterior resection	0 (0.0%)	24 (85.7%)

densities of ganglion cells in elderly and nonelderly rectosigmoid cancer groups (638.60 mm^{-2} and 705.00 mm^{-2}, respectively, $P > 0.05$).

4. Discussion

SV most commonly occurs in elderly men, and its etiology is multifactorial and controversial (3). Hirschsprung's disease (congenital aganglionic megacolon, the pathophysiology of which is described as the absence of ganglion cells in a bowel segment) is defined as a predisposing factor for the development of SV, and SV is observed more frequently in patients with this disease (3,12–15). Tan et al. (13) reported 10 infant cases of SV that occurred in patients with Hirschsprung's disease. Similarly, Venugopal et al. (14) and Erdener et al. (15) reported adult SV cases secondary to Hirschsprung's disease. In individuals with Hirschsprung's disease, the functional obstruction of the aganglionic segment gives rise to massive dilatation and hypertrophy of the proximal ganglionic sigmoid colon, predisposing individuals to SV (8,13). Similar to Hirschsprung's disease, acquired megacolon (in which, in contrast to Hirschsprung's disease, ganglion cells are present in the bowel wall, but the loss of coordinating contractions ensues due to damage to the ganglion cells) is also accepted as a predisposing factor for the development of SV (3,12,16–19). Ryan (16) reported 6 SV cases with acquired megacolon, and Harbrecht and Fry (17) showed a similar relationship in 3 cases. In individuals with acquired megacolon, colonic dysmotility due to the involvement of neural control leads to the dilatation and elongation of the sigmoid colon, causing SV (18,19).

Hirschsprung's disease and acquired megacolon may also change the clinical course in most SV cases (16). In addition, these diseases are known causes of the recurrence of SV, particularly in patients in whom nonoperative detorsion or limited bowel resection is performed (16–18,20–23). In a 6-patient series, Ryan (16) reported that the history was longer, the episodes were more severe, the nonoperative treatment was less effective, and recurrence was more frequent in such patients. Harbrecht and Fry (17) showed similar results related to the recurrence of SV in a 3-patient group. According to the reports of Morrissey and Deitch (18), Chung et al. (20), and Strom et al. (21), the explanation for the high recurrence rate is that these patients generally have abnormal colonic motility or atony, in addition to a residual dilated colon section proximal to the site of the sigmoid resection; thus, limiting the resection appears to represent a partial solution. Therefore, a subtotal (18,21) or total (16) colectomy is advocated as the primary choice in the treatment of SV patients with megacolon.

As observed, the relationships between congenital aganglionic or acquired megacolon and the occurrence, clinical presentation, and recurrence of SV are well described (13–17). In contrast, the role of the quantity of the ganglion cells has not been well documented, and the only study to date was performed by Furaya et al. (8) in 2005. These authors used neuron-specific enolase to detect ganglion cells by immunohistochemical staining in the both Meissner's plexus and Auerbach's plexus. Although Furaya et al. (8) reported a significantly lower average numerical density of ganglion cells in the SV group than in the nonvolvulus group when they counted the numerical density of the ganglion cells at 200× magnification in contiguous fields, they found no significant difference in the average numerical density of ganglion cells per unit area when they adopted another formula after 2-dimensional correction. Their assumption was that the bowel wall was uniformly stretched in 3 dimensions (8), and the major moot point was their use of a 2-dimensional counting technique in their study. We used a systematic random sampling method for each block, and we also analyzed each section using the Stereo Investigator software program. In addition, the coefficient error was under 0.05 in our study (Table 3). Otherwise, stereological methods, based on principles of geometry, statistics, and unbiased sampling methods, acquire quantitative data in histopathological studies, and their results do not change according to researchers or section areas (9). Thus, our 2-dimensional study and fractionator method is more reliable than it was before. Although Barbosa and Tafuri (24) demonstrated a decrease in the numerical density of ganglion cells in

Table 2. The mean numerical density of ganglion cells in Auerbach's plexus in the groups and the statistical significance.

Groups	Ganglion cells numerical density (mean ± SD)	Statistical significance
Sigmoid volvulus (n = 28)	140.70 ± 98.62 mm^{-2}	P = 0.005
Rectosigmoid cancer (n = 28)	669.42 ± 319.03 mm^{-2}	
Sigmoid volvulus, revolvulus (n = 6)	128.45 ± 82.53 mm^{-2}	P > 0.05
Sigmoid volvulus, nonrevolvulus (n = 22)	185.67 ± 137.75 mm^{-2}	
Sigmoid volvulus, 60 years of age and older (n = 14)	66.57 ± 81.96 mm^{-2}	P = 0.002
Sigmoid volvulus, under 60 years of age (n = 14)	214.86 ± 30.48 mm^{-2}	
Rectosigmoid cancer, 60 years of age and older (n = 15)	638.60 ± 353.36 mm^{-2}	P > 0.05
Rectosigmoid cancer, under 60 years of age (n = 13)	705.00 ± 268.48 mm^{-2}	

Table 3. Stereological details of the study for one section.

Stereological details	Sigmoid volvulus	Rectosigmoid cancer
Total markers counted	371	469
Number of sampling sites	73	79
Sampling grid area (XY, μm^2)	23,185	13,632
Coefficient error	0.05	0.04

hypertrophic colons above stenoses in an experimental study, this decrease was most likely secondary to bowel obstruction, and there are not enough available data to evaluate this relationship, particularly in patients with SV. Although based on the findings of the present study, the presence of a significantly lower numerical density of ganglion cells in the SV group relative to that in the control group is thought to be a predisposing factor in the development of SV, the cause-and-effect relationship is not determined, and further studies are needed. The present study also demonstrated that there was no significant difference between the ganglion cell numerical densities of the revolvulus and nonrevolvulus SV groups, similar to the results of Furaya et al. (8) In our study, when the numerical densities of ganglion cells of elderly patients were compared with those of nonelderly patients, the numerical density was found to be significantly lower in the elderly SV group, while there was no significant difference in RSC groups, and this result suggested that there may be a degenerative loss of ganglion cells due to advancing age in SV patients.

In conclusion, although the cause-and-effect relationship between the quantity of the ganglion cells and SV is not clear and further studies are needed, according to the results of the present study, in which we elected the control group from obstructive patients similar to the study group and we evaluated the intermuscular area to blind the possible effects of the bowel enlargement on the numerical density of the ganglion cells, we can argue that a decreased numerical density of ganglion cells may increase the likelihood of SV, and together with advanced age, a decreased numerical density of ganglion cell may be a predisposing factor for the development of SV.

References

1. Lal SK, Morgenstern R, Vinjirayer EP, Matin A. Sigmoid volvulus: an update. Gastrointest Endoscopy 2006; 16: 175–87.
2. Nuhu A, Jah A. Acute sigmoid volvulus in a West African population. Ann Afr Med 2010; 9: 86–90.
3. Raveenthiran V, Madiba TE, Atamanalp SS, De U. Volvulus of the sigmoid colon. Colorectal Dis 2010; 12: E1–E17.
4. Atamanalp SS, Öztürk G. Sigmoid volvulus in pregnancy. Turk J Med Sci 2012; 42: 9–15.
5. Bhatnagar BNS, Sharma CLN, Gupta SN, Mathur MM, Reddy DCS. Study on the anatomical dimensions of the human sigmoid colon. Clin Anat 2004; 17: 236–43.
6. Atamanalp SS, Öztürk G, Aydınlı B, Ören D. The relationship of the anatomical dimensions of the sigmoid colon with sigmoid volvulus. Turk J Med Sci 2011; 41: 377–82.
7. Atamanalp SS. Ileosigmoid knotting in pregnancy. Turk J Med Sci 2012; 42: 553–8.
8. Furaya Y, Yasuhara H, Yanagie H, Naka S, Takenoue T, Shinkawa H et al. Role of ganglion cells in sigmoid volvulus. World J Surg 2005; 29: 88–91.
9. Gundersen HJG, Bendtsen TF, Korbo L. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 1988; 96: 379–94.
10. Gundersen HJG, Jensen EB. The efficacy of systematic sampling in stereology and its prediction. J Microsc 1987; 147: 229–63.
11. Gundersen HJG, Bagger P, Bendtsen TF, Evans SM, Korbo L, Marcussen N et al. The new stereological tools: disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. APMIS 1988; 96: 857–81.
12. Avots-Avotins KV, Waugh DE. Colon volvulus and geriatric patient. Surg Clin North Am 1982; 62: 248–60.
13. Tan FLS, Tan YM, Heah SM, Seow-Choen F. Adult Hirschsprung's disease presenting as sigmoid volvulus: a case report and review of literature. Tech Coloproctol 2006; 10: 245–8.
14. Venugopal KS, Wilcox DT, Bruce J. Hirschsprung's disease presenting as sigmoid volvulus in a newborn. Eur J Pediatr Surg 1997; 7: 172–3.
15. Erdener A, Ulman I, Ozcan C, Genc K. A case of sigmoid volvulus secondary to Hirschsprung's disease. Pediatr Surg Int 1995; 10: 409–10.
16. Ryan P. Sigmoid volvulus with and without megacolon. Dis Colon Rectum 1982; 25: 673–9.
17. Harbrecht PJ, Fry DE. Recurrence of volvulus after sigmoidectomy. Dis Colon Rectum 1979; 22: 420–4.
18. Morrissey TB, Deitch EA. Recurrence of sigmoid volvulus after surgical intervention. Am Surg 1994; 60: 329–31.
19. Sonnenberg A, Tsou VT, Müller AD. The 'institutional colon': a frequent colonic dysmotility in psychiatric and neurologic disease. Am J Gastroenterol 1994; 89: 62–6.

20. Chung YFA, Eu KW, Nyam DCNK, Leong AFPK, Ho YH, Seow-Choen F. Minimizing recurrence after sigmoid volvulus. Br J Surg 1999; 86: 231–3.

21. Strom PR, Stone HH, Fabian TC. Colonic atony in association with sigmoid volvulus: its role in recurrence of obstructive symptoms. South Med J 1982; 75: 933–6.

22. Atamanalp SS, Ozturk G. Sigmoid volvulus in the elderly: outcomes of a 43-year, 453-patient experience. Surg Today 2011; 41: 514–9.

23. Cazador AC, Coll RF, Pujol FO, de Palol MP, Grillo AM, Romeu NG et al. Colonic volvulus and recurrence of volvulus: what should we do? Cirugia Espanola 2011; 89: 237–42.

24. Barbosa AJ, Tafuri WL. Ganglion cell number in hypertrophic colon above experimental stenosis. Braz J Med Biol Res 1983; 16: 165–9.