New Classification of Histochemical Staining Patterns of Acetylcholinesterase Activity in Rectal Suction Biopsy in Hirschsprung's Disease

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Abstract

Several previous studies have introduced classifications of Acetylcholinesterase (AChE) histochemical staining patterns in rectal suction biopsy performed in patients with Hirschsprung's disease. However, we introduce a new classification that is less complicated but shows the same age dependence as seen in previous studies. 135 rectal suction biopsies were submitted to histochemical staining for AChE activity and 88 specimens showed increased AChE activity. Therefore, we retrospectively analysed these 88 cases and could establish three patterns. Pattern I, presence of thick nerve trunks or coarse nerve fibers only in the muscularis mucosae and submucosa. This pattern was mainly seen in children aged 6 months or below. Pattern II, presence of abundant nerve fibers in all three layers of mucosa. This pattern was predominantly seen in children over 6 months of age. Pattern III, not predominant in any age group, showed positive nerve fibers in all three layers but, in one or more layers, the nerve fibers were sparse.

Upon comparison with previous studies, we could observe the same age-pattern relationship. Thus, we propose this method of classification as a new tool to classify AChE histochemical staining patterns.

Key word : Patterns of Acetylcholinesterase Activity, Rectal Suction Biopsy, Hirschsprung's Disease

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Demonstration of the increasing activity of AChE in rectal suction biopsies from the colon has proved to be an accurate diagnostic tool to diagnose Hirschsprung's disease. Since it was introduced in1972 by Meier-Ruge[1], this method has been adopted as a reliable diagnostic test world wide[2-9].

Many previous studies have established the relationship between the patterns of histochemical staining and the ages of children with Hirschsprung's disease[2-6]. De Brito and Maksoud[6] introduced the importance of the character of nerve fibers distributed in the lamina propria, muscularis mucosae and submucosa. That is, the dominant presence of thick nerve trunks or coarse nerve fibers in the muscularis mucosae and submucosa was related to younger children, especially newborns. In contrast, the presence of fine nerve fibers in the lamina propria and less coarse nerve fibers in the muscularis mucosae and submucosa was mostly found in older cases.

Although this classification was established simultaneously with other previous studies and was demonstrated to have a significant relationship with age, it is time consuming for examiners to discriminate fine nerve fibers from coarse ones or thick nerve trunks. This fact stimulated us to retrospectively review the AChE histochemical staining data in order to find a new classification equally effective but less complicated.

MATERIAL AND METHOD

Rectal suction biopsies were performed in 135 cases suspected of Hirschsprung's disease and the specimens were submitted to histochemical staining for AChE activity. The age of the patients ranged from 6 days to 21 years. We used the staining method modified from that proposed by Lake BD in1978[10]. Instead of mounting the specimens on blocks of animal liver, they were embedded in the OCT compound which had been cooled in the freezing microtome. We did not freeze the specimens in hexane maintained at −80°C and distilled water was used instead of tap water. In addition, the duration for treating specimens in the osmium tetroxide was reduced from 10 to 5 minutes in order to minimize precipitation. Finally, the specimens were not counter-stained with Carazzi's hematoxylin. Nevertheless, this modified technique was successful in demonstrating AChE activity.

After examining the staining patterns, 18 and 3 cases were excluded due to inadequate tissues (no identifiable submucosa) and anorectal, tissues, respectively. Moreover, 26 cases with normal AChE activity were also excluded. As a result, 88 cases with increased AChE activity were classified.

RESULTS

Of the 135 cases submitted to rectal suction biopsy, 88 showed increased AChE activity and had the distribution according to age as shown in Table 1.

Table 1. Age distribution of cases in this study.

Age group	No. of cases	Percentage
0 to 1 month	17	19
>1 month to 3 months	11	12.5
>3 months to 6 months	11	12.5
>6 months to 1 year	17	19
>1 year to 4 years	22	25
>4 years	10	12
Total	88	100

After analysis of the patterns of increased AChE activity, we classified the patterns of these 88 cases into three categories (or patterns). The classification was based on the distribution and density of AChE positive nerve fibers in the three layers of biopsy specimens : lamina propria, muscularis mucosae and submucosa.

Pattern I, thick nerve trunks or coarse nerve fibers or, even fine nerve fibers distributed in the submucosa and muscularis mucosae. In comparison, there were no positive nerve fibers in the lamina propria (Fig. 1B). Twenty-five cases were included in this pattern and had an age distribution as depicted in Table 2. In the majority of these cases (72%) the ages were not above 6 months, especially in the first age group (40%).

Pattern II, abundant thick nerve trunks and coarse or fine nerve fibers were present in all three layers (Fig. 1C). Forty-five cases were included in this pattern. As seen in Table 3, the majority of cases (71%) were over 6 months of age.

Pattern III, positive nerve fibers were found in all three layers but, in one or more layers.

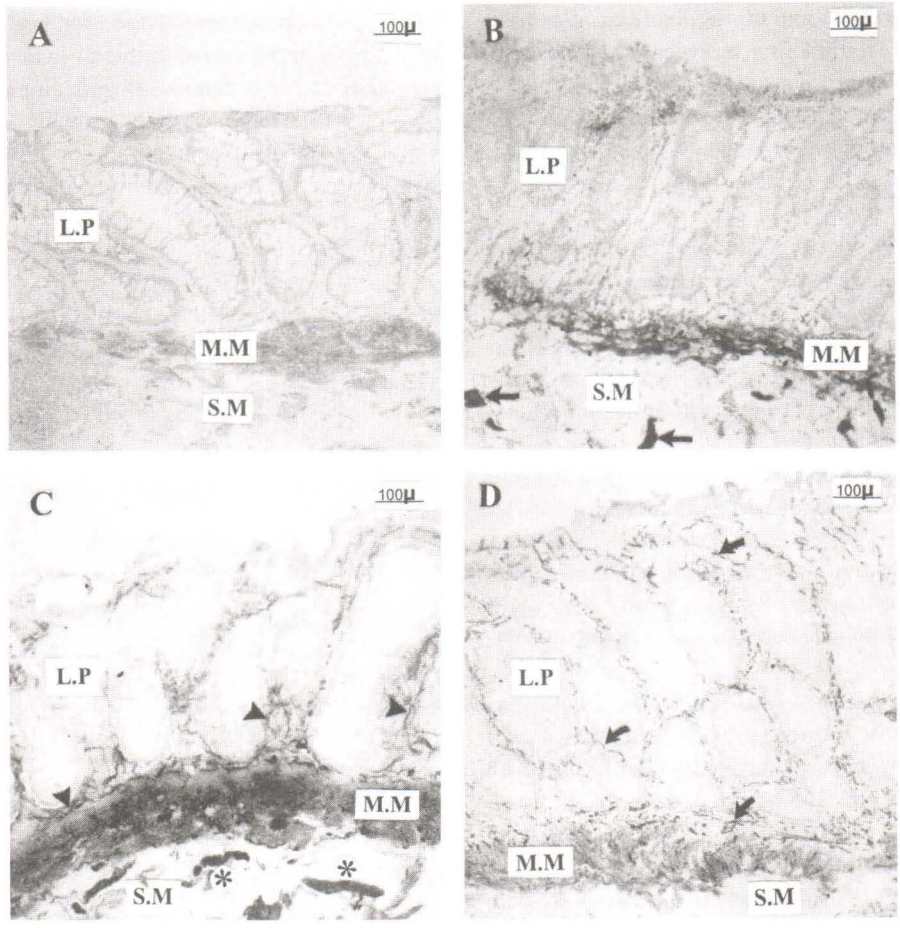

Fig. 1. Rectal suction biopsy stained for AChE activity.

(A) From a normal control. AChE is barely detectable in all three layers X 10.

(B) Pattern I of increased AChE activity. The muscularis mucosae shows increased activity and the submucosa also shows several thick nerve trunks (arrows). In contrast, positive nerve fibers are rarely observed in the lamina propria X 10.

(C) Pattern II. The increased AChE activity is striking thick nerve trunks in the submucosa (asterisks) and coarse and fine nerve fibers (arrowheads) in the upper two layers X 10.

(D) Pattern III. Prominent positive nerve is confined in the laminal propria and muscularis mucosae (arrows) here. No AChE-stained nerve fiber is found in thesubmucosa X 10.

Table 2. Age distribution in Pattern I.

Age group	No. of cases	Percentage
0 to 1 month	10	40
>1 month to 3 months	3	12
>3 months to 6 months	5	20
>6 months to 1 year	1	4
>1 year to 4 years	5	20
>4 years	1	4
Total	25	100

Table 3. Age distribution in Pattern II.

Age group	No. of cases	Percentage
0 to 1 month	5	11
>1 month to 3 months	3	7
>3 months to 6 months	5	11
>6 months to 1 year	13	29
>1 year to 4 years	13	29
>4 years	6	13
Total	45	100

Table 4. Age distribution in Pattern III.

Age group	No. of cases	Percentage
0 to 1 month	2	11
>1 month to 3 months	5	28
>3 months to 6 months	1	5
>6 months to 1 year	3	17
>1 year to 4 years	4	22
>4 years	3	17
Total	18	100

the positive fibers were sparse (Fig. 1D). Eighteen cases displayed histochemical staining in this pattern. The cases were equally distributed among all age groups and no majority could be established in any age group (Table 4).

DISCUSSION

We observed two age-related patterns of increased AChE activity. Pattern I was the pattern most commonly found in the 0-1 month age group (59%) (Table 5). This was characterized by thick nerve trunks or nerve fibers in the submucosa and muscularis mucosae and absence of nerve fibers in the lamina propria. Pattern II was mainly observed in older children with Hirschsprung's disease. Numerous thick nerve trunks or nerve fibers could be demonstrated in this pattern. According to Table 5, the cases displaying this pattern were most numerous among the three older age groups (76, 59 and 60%, respectively).

In contrast, with pattern III, we could not establish any age dependence as it included all staining patterns not compatible with pattern II or I.

Maybe, this pattern is part of pattern II, but the AChE activity was slightly increased in one or more layers. This minimal increase was probably due to less lack of ganglion cells and less proliferation of AChE positive nerve fibers. On the other hand, some cases showing nerve fibers in the submucosa and muscularis mucosae with slight fine nerve fibers in the lamina propria, were probably the forms progressing from pattern I. With reference to the age-dependent development proposed by DeBrito and Maksoud[6], we suppose this particular pattern to have evolved from pattern I.

Hence, from these two possible explanations, we could conclude there was an insignificant difference between the numbers of pattern III cases above and below 6 months of age (56 and 44%, respectively).

These results are compatible with those reported from previous studies. Chow, Chan and Yue[2] proposed their classification of histochemical staining and the staining patterns were designated as Type A positive and Type B positive. In comparison, our pattern I was a subgroup of Type B positive and showed the same age dependence, though these two patterns were not entirely identical. This correlation could also be observed between our pattern II and Type A positive.

Moreover,upon comparison with the patterns proposed by De Brito and Maksoud[6], our pattern I was quite similar to their pattern I and the results remained analogous. With pattern II also the criteria and results were compatible. However, our pattern III was not related to the age group between patterns I and II as proposed in their study. This pattern was dispersed among every age group and as a result, we could not establish an age group related to it.

In conclusion, our results showed a relationship between patterns and age groups partly similar to both studies mentioned above. But what is more important, our classification could be made more easily. Examiners only have to look for the presence or absence of AChE positive nerve fibers

Table 5. Distribution of cases in each age group according to three patterns.

	0-1 mo		>1-3 mo		>3 - 6 mo		>6 mo - 1 yr		>1 yr - 4 yr		> 4 yr	
		%		%		%		%		%		%
Pattern I	10	59	3	27	5	45.5	1	6	5	23	1	10
Pattern II	5	29	3	27	5	45.5	13	76	13	59	6	60
Pattern III	2	12	5	46	1	9	3	18	4	18	3	30
Total	17	100	11	100	11	100	17	100	22	100	10	100

and how densely the fibers are packed. It is unnecessary to discriminate between thick nerve trunks and fine or coarse nerve fibers, and more time can be saved during slide examination.

However, the relationship between these patterns and severity in clinical presentation requires further comparative studies.

SUMMARY

Our age-pattern relationship results confirmed the previous studies in AChE histochemical staining. The presence of AChE positive nerve fibers in the muscularis mucosae and submucosa, designated as pattern I, was mostly seen in children aged 6 months or below. Pattern II, filled with numerous positive nerve fibers in all three layers, was predominantly seen in children above 6 months of age. Lastly, with pattern III no correlation with age could be observed.

Moreover, the classification applied in this study had the advantage of saving time during slide examination.

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วิธีใหม่ในการจัดแบ่งรูปแบบการเพิ่มขึ้นของอะซิติลโคลินเอสเทอเรส ในชิ้นเนื้อที่ดูดตัดจากลำไส้ใหญ่ส่วนล่างของผู้ป่วยโรคเฮอร์ชปรุง

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มีหลายการศึกษาก่อนหน้านี้ได้เสนอวิธีการจัดแบ่งรูปแบบการเพิ่มขึ้นของอะซิติลโคลินเอสเทอเรสในชิ้นเนื้อที่ดูดตัดจากลำไส้ใหญ่ส่วนล่าง (Rectal suction biopsy) ของผู้ป่วยโรคเฮอร์ชปรุงมาแล้ว แต่คณะผู้วิจัยได้เสนอวิธีใหม่ในการจัดแบ่งเพื่อความสะดวกในการอ่านผลและยังคงมีความสัมพันธ์กับอายุเช่นเดียวกับการศึกษาก่อน ดังนั้นคณะผู้วิจัยจึงได้ รวบรวมผลของผู้ป่วย 88 ราย ที่มีการเพิ่มขึ้นของอะซิติลโคลินเอสเทอเรสจากทั้งหมด 135 ราย ที่ได้รับการดูดตัดชิ้นเนื้อจากลำไส้ใหญ่ส่วนล่างมาตรวจ จากการวิเคราะห์ผลดังกล่าว ทำให้สามารถจัดแบ่งรูปแบบการเพิ่มขึ้นของอะซิติลโคลิน-เอสเทอเรส ออกได้เป็น 3 รูปแบบคือ รูปแบบแรก จะพบเส้นประสาทในชั้น muscularis mucosae และ submucosa เท่านั้นแต่ไม่พบในชั้น lamina propria รูปแบบแรกนี้ส่วนมากพบในผู้ป่วยอายุไม่เกิน 6 เดือน รูปแบบที่สองคือ พบเส้นประสาทเป็นจำนวนมากทั้ง 3 ชั้น โดยส่วนมากจะพบในผู้ป่วยอายุมากกว่า 6 เดือน และรูปแบบที่สามจะพบเส้นประสาททั้ง 3 ชั้น แต่มีบางชั้นที่พบปริมาณเส้นประสาทได้น้อย ซึ่งรูปแบบนี้จะกระจายอยู่ในทุกกลุ่มอายุของผู้ป่วยใกล้เคียงกัน

จากการเปรียบเทียบวิธีการจัดแบ่งแบบใหม่กับการศึกษาต่างๆ ก่อนหน้านี้ พบว่าผลนั้นเป็นไปในทางเดียวกัน โดยมีลักษณะความสัมพันธ์ระหว่างรูปแบบกับอายุของผู้ป่วยเหมือนกัน คณะผู้เขียนจึงเสนอวิธีการจัดแบ่งใหม่นี้ในการจัดแบ่งรูปแบบการเพิ่มขึ้นของอะซิติลโคลินเอสเทอเรสในชิ้นเนื้อที่ดูดตัดจากลำไส้ใหญ่ส่วนล่างของผู้ป่วยโรคเฮอร์ชปรุง

คำสำคัญ : รูปแบบการเพิ่มขึ้นของอะซิติลโคลินเอสเทอเรส, การดูดตัดชิ้นเนื้อจากลำไส้ใหญ่ส่วนล่าง, โรคเฮอร์ชปรุง

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