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Immunohistochemical localization and distribution of Cajal cell in the intestine of rabbit

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Abstract

A total of ten male local rabbits were used, a one centimeter was taken from the small intestine as well as from the colon. H&E and Masson's Trichrome stains were used to stain the slides. Location, shape and size, number/section in each segment of the intestine, and surface area of nerve plexuses/ µm² were studied. The CD117 was used to express the interstitial cell of Cajal (ICC). Myenteric plexus (MP) morphology and distribution in the intestine provide interpretation for intestinal peristaltic motility. The current study showed that Auerbach's plexuses (AP) had an oval or elliptical shape in the duodenum and ileum while appearing spherical in the jejunum and located either between two layers of tunica muscularis or within the internal circular muscular layer (ICML). In contrast, the AP of the colon appeared as a line of neurons in the exact location along the whole wall. ICC in this study showed high expression in the colon, whereas their expression ranged from mild to moderate in three segments of the small intestine. In conclusion, most ICC were found distributed in different locations throughout the wall of the small intestine, whereas they concentrated near MP and within ICML of the colon's wall.

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Introduction

The barrier of the alimentary canal is brilliant with a compound neuronal system identified as the enteric nervous system (ENS). ENS is liable for alimentary canal movement shapes, satisfactory digestive discharge of diverse enzymes, electrolytes, and mucus, conditional on the morpho-physiological area, and coordinating the immune and protective purposes of the alimentary canal (1). The regulation of alimentary canal motility exists in stability among hormonal, myogenic, and neuronal activities. Once remote, furthermost, but not totally, areas of the digestive canal produce flowing automated movement without neuronal or hormonal stimulus. The minute electrical records are completed from the myogenically muscle sheets of various areas of the alimentary canal, flowing influenced by depolarization, named slow waves (SW), which were documented since the smooth muscle (SM) cells. Slow movement contractions are generated individually, and even though the contractions are obstructed by managers that clog (SM) voltage-dependent Ca^{+2} canals, the amplitudes and period sequences of SW are small. Subsequently SW was easily documented from SM cells, and it was assumed that these waves were (2-6). Consequently, it became known that the muscular barrier of the alimentary canal, comprising SM cells, contains a population of specified cells that lack contractile components. These cells are called Interstitial Cells of Cajal, and it was recommended, according to the electrophysiological works, that these cells might be pacesetter cells (7-9). Overall, ICC can be separated into two collections. In maximum areas of the alimentary canal, a skinny sheet of ICC makes a cellular web (10,11). ICCs are distributed through the alimentary canal in 2 plexuses: the Auerbach's (or myenteric) plexus, which subsumes ganglia that are located among the circular and longitudinal muscle sheet, and the Meissner's (or submucosal) plexus, that is situated among the internal muscular sheet of the submucosal and the mucosal layer (12,13). The Auerbach's

plexus regulates muscle contraction, while the Meissner's plexus regulates secretory action and blood flow (14).

The present work aims to understand the histology, histochemistry, and immunohistochemistry distribution of ICC throughout the rabbit intestine.

Materials and methods

Ethical approve

Ten male local rabbits (*Oryctolagus cuniculus*) were conducted in the current study under the local Ethics Committee of the College of Vet Med at the University of Mosul (UM.VET. 2023.066 on 15/10/2023).

Animals of the study

The rabbits were kept in separate cages at a temperature 23±2°C below a condition (12:12h) light: dark sequence and delivered with free admission to food (ad libitum) and water. Animals were reserved under the care of condition to ensure good healthy condition before their euthanasia and subsequent dissection.

Euthanization and sample collection

All animals were euthanized with sodium pentobarbital and ketamine HCl (15,16). The characteristic samples (1 cm) were reserved from the small intestine and colon. The small and large intestine samples were cleaned free of intra-luminal fillings and sited directly in ten percent neutral buffered formalin for 48 hours. Predictable histological processing methods were conducted to obtain histological slides of 5µm thickness (17-19). H&E and Masson's Trichrome stains were used to stain the slides (20). The stained sections were examined using a light microscope (Olympus-CX21) equipped with a digital microscope camera (Omax-18MP) and provided with picture analysis software (Toup view). Location, shape size, and number/section in each segment of the intestine and surface area of nerve plexuses/ µm² were studied with camera software. To study the distribution and location of ICC in all segments of rabbit intestine specimens, the c-Kit (CD117), 1:500 (DAKO, Glost) was used to express ICC (21,22).

Statistical analysis

A computerized package of Sigma stat (V13.0/ SYSTAT software) was used to complete the statistical examination. The records offered as (Means ± SE) were evaluated by (ANOVA), and the significance was set at $P \leq 0.05$. The variances between the intestinal parts studied were resolute using Duncan's multiple-range test (23).

Results

The Auerbach's nerve plexuses in the duodenum were in two locations: between two sheets of tunica muscularis and within the internal circular layer of the tunica muscularis, and their shape was varied between oval to elliptical shapes. While in the jejunum, most AP were located only between two layers of tunica muscularis, and most of them were spherical in shape and contained each plexus with two neurons. In the ileum AP was located just between two layers of tunica muscularis, and most had an elongated shape. AP was positioned among two sheets of tunica muscularis of the colon, and most of them had an elongated shape, and few of them had a spherical shape and arranged as a line of neurons between the two layers of tunica muscularis, supplied by a large number of myenteric plexuses; and approximately surrounded all the wall of the colon (Figure 1). The interstitial cells of Cajal were demonstrated poorly with hematoxylin and eosin stain. In contrast, in sections stained with Masson's trichrome stain, they appeared clearly with a dark brown to black color, and their processes extended obliquely from the periphery of Auerbach nerve plexuses to the submucosal Cajal cells across the internal circular layer of tunica muscularis (Figure 2).

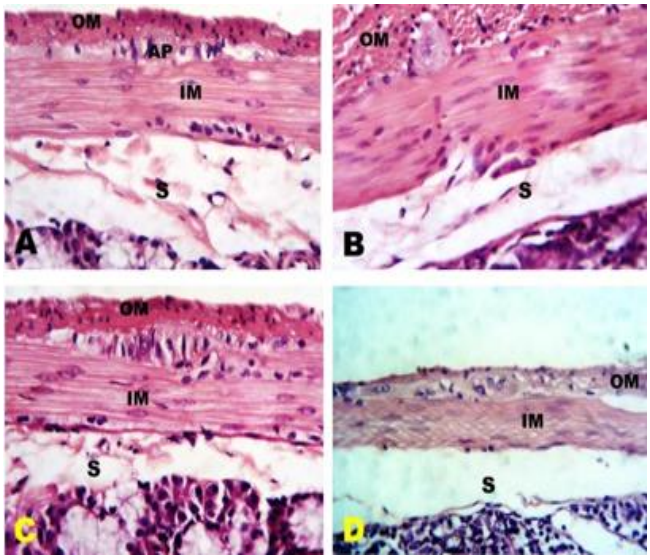

Figure 1: Histological sections of different segments of intestinal wall; (A) duodenum, (B) jejunum, (C) ileum, (D) colon showing submucosa (S), internal circular sheet of tunica muscularis (IM), outer sheet of tunica muscularis (OM), Auerbach's nerve plexus (AP). H&E. 400x.

Immunohistochemistry results

Immunohistochemistry staining with CD117 was conducted to investigate the shape and location of ICC. Positive cells appeared with a dark brown color. The shape of cells appeared either oval or elliptical to fusiform. Most ICC possess delicate processes that extend across the smooth muscle fibers of tunica muscularis toward the submucosa, and most of these processes are oriented perpendicularly or obliquely to the muscle fibers. Generally, ICC is found to be located in the perimyenteric plexus, within an internal

circular layer of tunica muscularis, submucosal at the border line between tunica submucosa and tunica muscularis, and at the sub-serosal layer. In the duodenum, most of the ICC was found in the submucosal layer, and few numbers were found within an internal circular layer of the tunica muscularis and perimyenteric plexus; most of the ICC in the duodenum had an elliptical shape with few processes, and some cells had no processes (Figure 3).

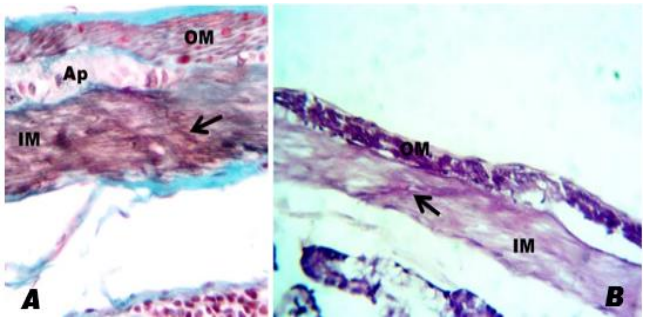

Figure 2: Histological sections of (A add on photo) jejunum, (B add on photo) colon wall; showing processes of Cajal cell (arrows) that extend obliquely in the internal circular sheet of tunica muscularis (IM) from Auerbach's nerve plexus (AP), outer sheet (OM). (A) Masson's trichrome stain, (B) H&E. 400x.

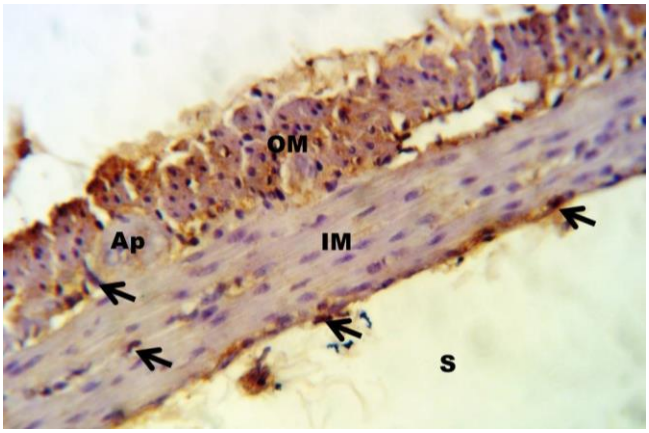

Figure 3: Histological sections of duodenum, showing submucosa (S), internal circular sheet of tunica muscularis (IM), outer sheet of tunica muscularis (OM), Auerbach's nerve plexus (AP) and Cajal cells (arrows). IHC c-Kit. 400x.

In the jejunum, (ICC) were distributed in different locations within the wall of the jejunum, where most were found in the submucosal layer and equally distributed in the peri myenteric plexus and sub-serosal type; most of the cells appeared oval in shape with few processes (Figure 4). Whereas, ICC within an internal circular layer of tunica muscularis was absent. In the ileum, most ICC were found in the sub-serosal location and submucosal layer, and very few numbers were found in perimyenteric plexuses and within an internal circular layer of tunica muscularis and had an oval shape (Figure 5). While in the colon, most ICC were found in two main locations, which are the perimyenteric plexus and within an internal circular layer of tunica muscularis, and very few numbers were found in the submucosal and sub-serosal layer; most of the ICC of the colon appeared elongated or star shape with prominent processes. ICC showed high expression in the colon, whereas their expression ranged from mild to moderate in three segments of the small intestine (Figure 6).

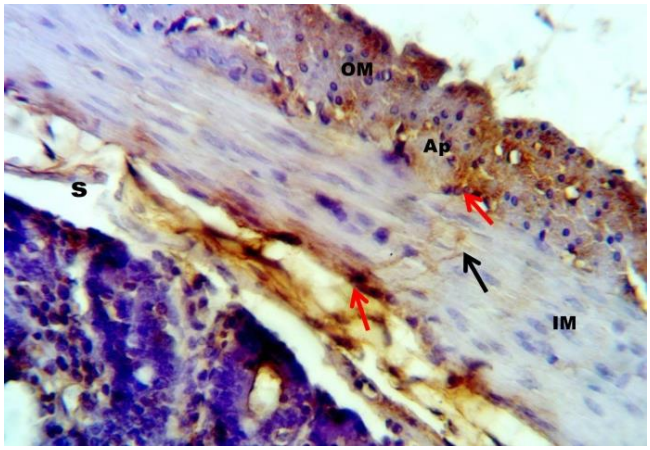

Figure 4: Histological sections of Jejunum; showing submucosa (S), internal circular sheet of tunica muscularis (IM), outer sheet of tunica muscularis (OM), Auerbach's nerve plexus (AP), Cajal cells (red arrows) with its processes (black arrows). IHC c-Kit. 400x.

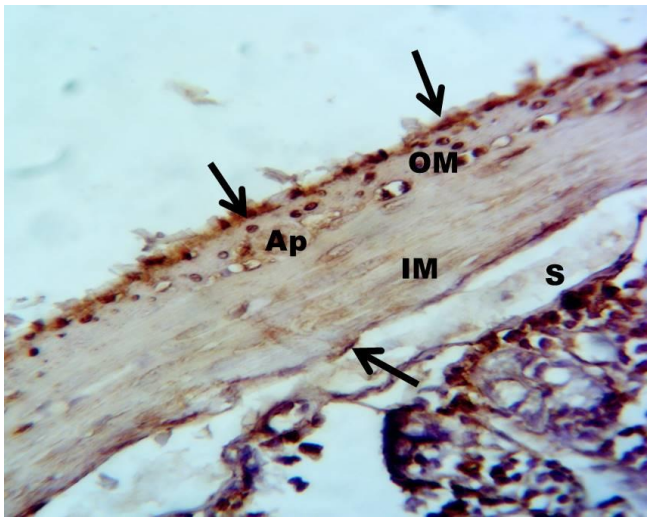

Figure 5: Histological sections of Ileum; showing submucosa (S), internal circular sheet of tunica muscularis (IM), outer sheet of tunica muscularis (OM), Auerbach's nerve plexus (AP), Cajal cells (arrows). IHC c-Kit. 400x.

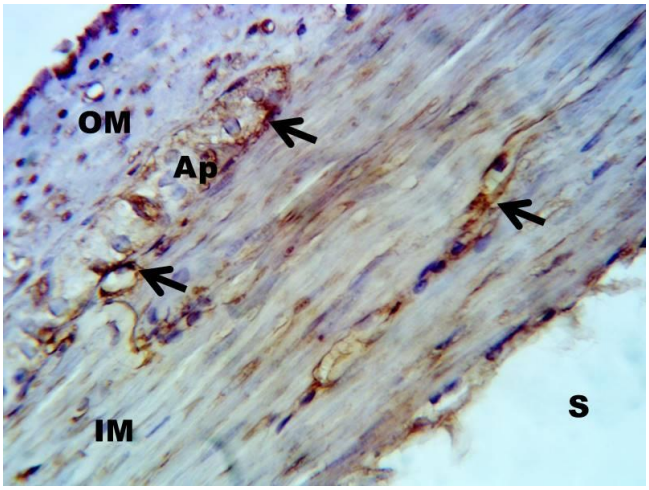

Figure 6: Histological sections of colon, showing submucosa (S), internal circular sheet of tunica muscularis (IM), outer sheet of tunica muscularis (OM), Auerbach's nerve plexus (Ap), Cajal cells (arrows). IHC c-Kit. 400x.

Morphometric analysis of Auerbach's plexuses

The histomorphometric measurements of Auerbach's plexuses in the small and large intestine wall showed that the highest number of myenteric plexuses per section was recorded in the colon and jejunum, while the lowest number was recorded in the duodenum. On the other hand, the highest surface area of each plexus was recorded in the colon and ileum, and the lowest surface area of each plexus was found in the jejunum. Furthermore, the correlation between each nerve plexus's number and surface area showed that the highest surface area per section was found in the colon, and the lowest surface area was recorded in the duodenum (Table 1).

Table 1: Micromorphometric measurements of Auerbach's nerve plexuses in different segments of rabbit intestine

Segments of rabbit intestine	Auerbach's nerve plexus	
	Number (section)	Surface area (µm²/section)
Duodenum	22±0.9 a	23760.2±314.7 a
Jejunum	43±1.3 b	29076.3±1108.3 b
Ileum	29±0.8 c	43954.6±899.4 c
Colon	45±1.5 d	74642.6±1257.5 d

Different letters mean there is a significant difference among intestinal segments at P≤0.05.

Discussion

Myenteric nerve plexus morphology and intestine distribution propose or provide interpretation for intestinal peristaltic motility (24,25). The current study showed that AP had an oval or oval elongated shape in the duodenum and ileum while appeared spherical in the jejunum and located either between two layers of tunica muscularis or within the internal circular muscular layer, whereas the AP of the colon appeared as a line of neurons in the same location along the whole wall. Al-Saffar (26) mentioned that the most well-developing myenteric plexus was in the colon, while the least was in the caecum. Similarly, Björnhag (27) cited Auerbach's nerve plexuses in Guinea pig intestines as having different sizes and shapes (spherical or elongated), mainly in tunica muscularis. These results propose different neuronal mechanisms and regulators above the motility of separate intestinal divisions, thus giving an essential thought ENS in rabbits.

This study showed that the occurrence of Auerbach's nerve plexuses in the colon of rabbits appeared higher than those of the small intestine, and this may be necessary for the effectiveness of the colonic separation mechanism that permits rapid conveyance of the less digestible food elements through the digestive tract. In contrast, at the same time, they keep micro-organisms and easily digestible food elements in their caecum for an adequately long time for microbial fermentation and reproduction to occur (28).

A main restriction occurs in the scarcity of revisions SW of the colon in all species of mammals, where SW have been deliberate briefly only in the opossum, dog, and cat but not in local breed rabbits. The present work results sustain the impression of current Electron Microscopic research in the human colon (8). Our study was designed to understand if ICC density and distribution match the importance of SW in the small intestine and colon, which supports the impression that ICC plays a role in generating SW and later in the direction of peristaltic movement.

ICC in this study showed high expression in the colon, whereas their expression ranged from mild to moderate in three segments of the small intestine, and this result explains the importance of the density of these cells in the colon of rabbits. Most of the interstitial cells of Cajal were found distributed in diverse locations through the wall of three segments of the small intestine, whereas they concentrated near the myenteric plexuses and inside the internal circular muscular sheet of the wall of the colon; these results come inconsistent with that of López-Pingarrón (11) in human.

In this study, ICC in the small intestine had an oval-to-oval elongated shape with few processes. While the ICC of the colon appeared elongated or star-shaped with prominent processes, these outcomes were consistent with that of Iino (3) and Kim (29), whose found in their study that ICC correlated to Auerbach's plexus was multipolar and existing in the formula of a network. Morphologically, the Interstitial cell of Cajal was a combination of bipolar and stellate formed, and at the second-colic connection, popular ICC in the circular muscle sheet was bipolar in the form in the prenatal bovine intestine, but in the adult age, they performed stellate-shaped. This study's outcomes would be to sustain the knowledge that ICC is complicated in the compeers of

SW and henceforth in the directive of rhythmic peristalsis (8).

ICC significantly performs GI motility by facilitating enteric transmission (30). The cell plain morphology (bipolar and stellate), location (sub-muscular, intra-muscular, myenteric, and sub-serosal), and organization for each sub-kind of Interstitial cell of Cajal are principally resolute by their interactions with local nerve plexuses, and the regularity of networks among ICC themselves or their principal purposes (pacemakers, neuro-muscular, neuro-transmission, and mechano-reception) (31,32). The myenteric plexus is chiefly accountable for the controller of muscle contraction, while the submucosal plexus controls secretory purposes and blood flow directives (11,33,34).

Conclusion

In conclusion, most ICC were found distributed in different locations throughout the wall of the small intestine, whereas they concentrated near MP and within ICML of the colon's wall.

Acknowledgment

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Conflict of interest

The authors declared they have no competing interests.

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التعبير النسيجي الكيميائي المناعي لخلايا كاجال الخلالية وتوزيعها في أمعاء الأرنب

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الخلاصة

تم استخدام عشرة ذكور أرانب محلية، تم أخذ واحد سنتمتر من الاثني عشر والصائم واللفائفي وكذلك من القولون. تم صبغ الشرائح بصبغة الهيماتوكسيلين والايوسين وصبغة ماسون ثلاثية الألوان. تمت دراسة الانتشار والشكل والحجم والعدد لكل مقطع في كل جزء من الأمعاء؛ وقياس مساحة سطح الضفائر العصبية/ مايكرومتر مربع. تم استخدام عنقود التمايز ١١٧ للتعبير المناعي عن الخلايا الخلالية لكاجال. توفر شكل الضفيرة المعوية وتوزيعها في الأمعاء تفسيرًا للحركة الدودية للأمعاء. أظهرت الدراسة الحالية أن ضفائر أورباخ لها شكل بيضاوي أو بلحى في الاثني عشر واللفائفي بينما ظهرت بشكل كروي في الصائم. وتقع إما بين طبقتين من الغلالة العضلية أو داخل الطبقة العضلية الدائرية الداخلية، في حين ظهرت ضفائر أورباخ للقولون كخط من الخلايا العصبية في نفس الموقع على طول الجدار بأكمله. أظهرت الخلايا الخلالية للكاجال في هذه الدراسة تعبيرًا عاليًا في القولون، في حين تراوح تعبيرها من خفيف إلى متوسط في الثلاثة أجزاء من الأمعاء الدقيقة. في الختام، تم العثور على معظم الخلايا الخلالية للكاجال منتشرة في مواقع مختلفة من جدار الأمعاء الدقيقة في حين أنها تركزت بالقرب من الضفيرة المعوية وداخل الطبقة العضلية الدائرية الداخلية من جدار القولون.

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