Age-Related Variations in Enteric Glial Cells: A Comprehensive Microscopic Analysis of the Human Colon

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ABSTRACT

Background: Constipation and other lower intestinal disorders are more common in the middle-aged population. According to recent research, enteric glial cells (EGCs) may have an impact on colonic motility. Little is known about how ageing impacts EGCs in the human colon. This study aims to compare the morphology of EGCs in the colons of young and middle-aged individuals.

Objective: To study the age-related morphological variations in the EGCs of the myenteric plexus in human transverse colon.

Materials and Methods: Colon specimens from 11 deceased individuals were obtained from a mortuary and categorized into two age groups: Group 1 (Young, n = 6) and Group 2 (Middle-Aged, n = 5). Immunohistochemistry for Glial Fibrillary Acidic Protein (GFAP) and routine staining were performed. Both qualitative and quantitative evaluations were conducted.

Results: In the middle-aged group 2, vacuolization was observed between Myenteric Ganglia (MG), and myenteric neurons appeared more scattered compared to the young group 1. The number of myenteric neurons and EGCs decreased with increasing age. The mean count of EGCs per MG and per mm² of ganglionic area was significantly higher in group 1 (young) as compared to group 2 (middle-aged). The MG density, expressed relative to the thickness of the inner circular muscle, was significantly greater in group 1 (young).

Conclusion: There is a significant decrease in the number of EGCs with advancing age, along with notable morphological changes. These changes may contribute to various gut motility disorders observed in the middle-aged, impacting their quality of life.

KEYWORDS

enteric nervous system; gastrointestinal; myenteric ganglia; myenteric neurons; immunohistochemistry

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INTRODUCTION

Humans with advanced age are more likely to experience disorders such fecal impaction, constipation, and incontinence (1, 2). The gastrointestinal (GI) tract motility of the middle-aged can be affected by a variety of factors, including diet and exercise level (3) and the use of one or more prescription drugs – NSAIDs, calcium-channel antagonists, and calcium supplements (4). Human physiological and pharmacological research (5–8) also points to a decrease in afferent nerve and neuromuscular functions in the ageing colon, which is at least partially linked to "inflammageing" (9) and an increase in the expression of post-mitotic cellular senescence-like activity within enteric neurons (10).

Enteric Nervous System (ENS) is an intricate division of the peripheral nervous system (PNS) which is embedded in the wall of the gut. It exhibits plasticity and undergoes changes with ageing. The neurons and enteric glial cells (EGCs) are present in ganglia and are placed in the form of two major plexuses, the myenteric and the submucosal plexuses (11). The Myenteric Plexus (MP) lies between inner circular and outer longitudinal layers of smooth muscle of gut (12). Research on how ageing affects intestinal functions in humans has primarily looked at extrinsic, enteric, and muscular neurons (see above), but little is known about the existence or roles of EGCs as people age. Apart from their active functions in regulating several aspects of GI function (13) like mucosal permeability, EGCs surround neuronal cell bodies and processes, facilitating functional communication between EGCs and neurons (14). Moreover, EGCs have been implicated in the regulation of GI motility (15, 16), the provision of immunological support (17), and sufficient plasticity to generate new neurons or replace dead neurons (18).

It has been documented earlier that age-related changes in the ENS are seen such as neurodegeneration in colon as early as the fourth year of age in humans (19). It was also found that there was about 37% reduction in neurons between the ages of 20 to 35 and 65 years of age in humans. Animal model-based studies have been suggested that 40% neuronal loss in distal colon of the guinea pig at the age of 27 months, 60% loss of neurons in colon of rat at 6 and 24 months and 50–60% loss in distal colon of the mouse at 3 and 12 months (20). In rodent studies, the reduction of EGCs has been found to be proportional to the reduction of myenteric neurons (21). Loss of EGCs causes neuronal degeneration (22). Thus, neuronal integrity is maintained by EGCs by providing structural support, releasing neurotrophic factors and ensuring a protective environment. The EGCs are associated with many gut related disorders (23).

Currently, there is no universal marker that identifies all EGC populations in the GI system. The sex-determining region Y (SRY)-related HMG-box (Sox) 10 gene, a key marker for neural crest cell progenitors in the ENS, is the most recent marker that labels most, but not all, EGC populations (24). Additionally, EGCs are known to express the intermediate filament glial fibrillary acidic protein (GFAP) and the calcium-binding protein S100. To evaluate the impact of ageing on EGCs, it's crucial to identify distinct functional EGC subpopulations within the colonic wall sublayers. Currently recognized EGC populations based on morphology and location include: Type I: Intraganglionic, residing within the ganglia in the MP and submucosal plexus, Type II: Extraganglionic, located in the interganglionic fiber tracts connecting myenteric ganglia, Type III: Found in the mucosal region, Type IV: Located in the intramuscular layer (25, 26). Few studies have been done on the morphology of the EGCs and scant literature is there on ageing human subjects. Consequently, we looked into how ageing affected the morphology of EGCs (anti-GFAP) in the MP.

MATERIALS AND METHODS

The human transverse colon (n = 11) was collected from the mortuary of the Department of Forensic Medicine and Toxicology (Ethical clearance number IECPG-93/22.03.2017). All tissues were from males. Samples were obtained from cases of suicide and road traffic accidents (Table 1) with relevant medical and personal histories collected from relatives. The age and cause of death were determined from case sheets. Samples with known GI disorders or histories of alcohol and drug abuse were excluded. Most specimens were procured within 8 hours of death and washed before being preserved in 4% paraformaldehyde. The tissues were stored in the same solution at 4°C for further processing. A 4 cm segment of the transverse colon was resected and maintained in this preservative until subsequent analyses. For each tissue block, 3 serial sections were taken (each 5 µm thick). From each section, we captured 5–8 high-resolution images covering the MP across the entire circumference of the transverse colon segment. The average length of the myenteric plexus examined per sample was 12–15 mm, measured along the inner circular muscle.

Tab. 1 Parameters of postmortem cases.

S. No.	Age (years)	Sex	Cause of death
1	48	M	Hanging
2	42	M	Road traffic accident
3	17	M	Hanging
4	24	M	Hanging
5	30	M	Hanging
6	12	M	Hanging
7	20	M	Hanging
8	34	M	Hanging
9	51	M	Hanging
10	60	M	Myocardial Infarction
11	65	M	Myocardial Infarction

HISTOLOGICAL ANALYSIS METHODS

Hematoxylin and Eosin Staining: Paraffin sections were stained to visualize gut layers in the myenteric plexus (MP).

Glial Fibrillary Acidic Protein (GFAP) Immunohistochemistry: Cryosections were stained to visualize enteric glial cells (EGCs) in the MP, using a rabbit-derived GFAP antibody (Cell Signaling, 1:400).

Post-fixation, 4 cm longitudinal samples were dehydrated through graded alcohols, cleared in cedarwood oil, and embedded in paraffin. Paraffin blocks were sectioned at 7 µm using a microtome, floated in warm water, and mounted on glass slides coated with egg albumin and thymol. After air drying, slides were stained with hematoxylin and eosin.

IMMUNOHISTOCHEMISTRY

After fixation for 2 hours at 4 °C, longitudinal tissue slices were washed with chilled 0.1M phosphate buffer and cryoprotected in 15% sucrose for 3 hours and 30% sucrose for 8 hours at 4 °C. The tissues were then embedded in optimal cutting temperature compound and sectioned at 12 µm thickness using a LEICA cryostat microtome. The sections were mounted on 1% gelatin-coated glass slides [1% w/v gelatin, 0.01% w/v $Cr(SO_4)_2.12H_2O$] and air-dried, then stored at –20 °C for immunohistochemistry.

For antigen retrieval, Triton X was used, followed by quenching with methanol and hydrogen peroxide. Blocking was performed with 10% Normal Goat Serum (NGS) in 0.1M PBS-Tx for 2 hours. Sections were incubated with primary antibodies (anti-GFAP, Cell Signaling, 1:400) and secondary antibodies (ABC Kit, 1:10,000 dilution). Visualization was achieved using a DAB substrate kit. Finally, sections were dehydrated, counterstained with hematoxylin, and mounted with DPX coverslips

MORPHOMETRIC ANALYSIS

For morphometric analysis, sections were examined using a Nikon Eclipse 90i light microscope, and images were captured. These images were analyzed with ImageJ – Fiji software (National Institutes of Health, available at http://imagej.nih.gov/ij/). Volume density, defined as the volume of EGCs per unit volume of the MP, was measured using this software.

For quantitative analysis the samples were divided into two groups:

Group 1; Young (n = 6: 12, 17, 20, 24, 30, 34yrs) – lower age group <40yrs

Group 2; Middle-aged (n = 5: 42, 48, 51, 60, 65yrs) – middle age ≥40yrs

Parameters analyzed:

- Number of EGCs per myenteric ganglion. Glial cells were counted only when a clearly visible nucleus was present, and the surrounding cytoplasm was intact for more than half of the cell's circumference, consistent with established stereological criteria (42).
- Mean count of EGCs per mm^2 of ganglionic area.
- Myenteric fraction: The percentage of area occupied by the myenteric ganglia (neurons, EGCs and nerve fibers) with respect to the inner circular muscle was calculated with the help of Grid Cycloid Arc plugin in ImageJ software. The myenteric fraction per inner circular muscle was calculated over a standardized 10–mm length of the muscularis externa in each section. This length was selected because it consistently captured 2–3 myenteric ganglia per region.

STATISTICAL ANALYSIS

Statistical analysis was conducted with data presented as mean ± SEM using SPSS software, with assistance from the biostatistics department. The Mann-Whitney test was employed for comparing non-parametric data between groups. A probability level of ≤0.05 was considered statistically significant.

RESULTS

MICROSCOPIC FEATURES

Hematoxylin and eosin staining:

Myenteric ganglia (MG) were located between the two layers of the muscularis externa: the inner circular (IC) and outer longitudinal (OL) layers (Figure 1) across all age groups. The shape, size, and arrangement of MG varied with age in the human transverse colon. In younger individuals (12–30 years), MG appeared elongated (Figure 1 & 4). Elongated MG were also observed in middle-aged individuals (51–65 years), although the number of neurons and EGCs was significantly lower (Figures 4, 5). In middle-aged individuals (34–48 years), MG appeared spheroid (Figure 2). In younger ages, MG was continuous, but from middle age onwards, spaces appeared, making MG seem separated (Figure 2). The size of MG appeared reduced in middle-aged individuals (51–65 years) (Figures 10–13).

Changes in the shape, size, and distribution of myenteric neurons were observed with age. Myenteric neurons were either irregular or spherical to oval in shape (Figure neurons exhibited a horny profile. In younger individuals, myenteric neurons appeared smaller (Figure 1, 3), while in middle-aged individuals, they appeared larger (Figure 3). In middle-aged, myenteric neurons were predominantly located peripherally within the MG (Figure 3). The number of myenteric neurons and EGCs was observed to decrease starting from 42 years onwards (Figure 2, 3, 4, 5).

IMMUNOHISTOCHEMISTRY (IHC) FOR GFAP

GFAP is the primary marker for EGCs, and its expression in gut tissue confirms the presence of EGCs. IHC staining of cryo-sectioned transverse colon showed remarkable expression of GFAP in EGCs within the MG. Most ganglionic segments exhibited GFAP immunoreactivity, confirming the presence of a large number of EGCs. The non-reactive (unstained) portions of MG indicate a lack of GFAP expression. The GFAP expression patterns revealed well-developed small ganglionic plexuses between the IC and OL layers of the muscularis externa (Figure 6). Based on GFAP expression patterns, it was observed that the MG were highly populated with EGCs in the human transverse colon (Figure 6). EGCs were also distributed at the periphery of MG. In middle-aged individuals, no clear grouping of EGCs into distinct ganglia was found (Figure 9).

From 12 years onwards, the number of MG with EGCs increased with age until 34 years (Figure 7, 8). From 42 years onwards, the number of EGCs decreased with age (Figures 8, 9). In younger ages (12–24 years), MG appeared continuous and elongated (Figure 7). In middle age (30–42 years), MG appeared spheroid with spaces between them (Figure 8). In middle-aged individuals (51 years and above), MG again appeared elongated but with extensive spacing and significantly less expression compared to younger ages (Figure 9).

The number of EGCs and their processes increased with age until 34 years (Figure 7) and decreased from 42 years onwards (Figure 8, 9). The minimum expression of GFAP was observed at 65 years (Figure 9). No significant changes in the size and shape of EGCs were observed with increasing age. The bodies of EGCs were irregular in shape with many processes, which decreased in ageing MG. Minimal processes were observed in middle-aged individuals (Figures 8, 9). The irregular shape of EGC bodies was consistent across all ages in humans.

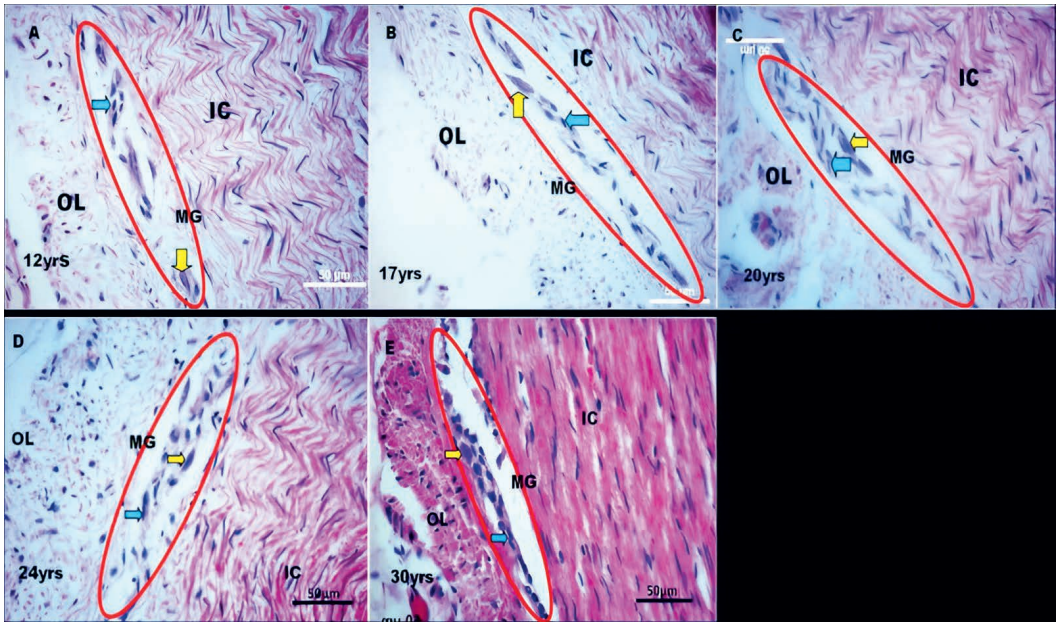

Fig. 1 Photomicrographs (40×) of Hematoxylin and Eosin-stained transverse sections of the human transverse colon from individuals aged 12 years (A), 17 years (B), 20 years (C), 24 years (D), and 30 years (E). Myenteric ganglia (MG) (red circle) are located between the inner circular (IC) and outer longitudinal (OL) muscle layers. Myenteric neurons (yellow arrows) and enteric glial cells (EGCs) (cyan arrows) are identifiable within the ganglia. Scale bar: 50 µm.

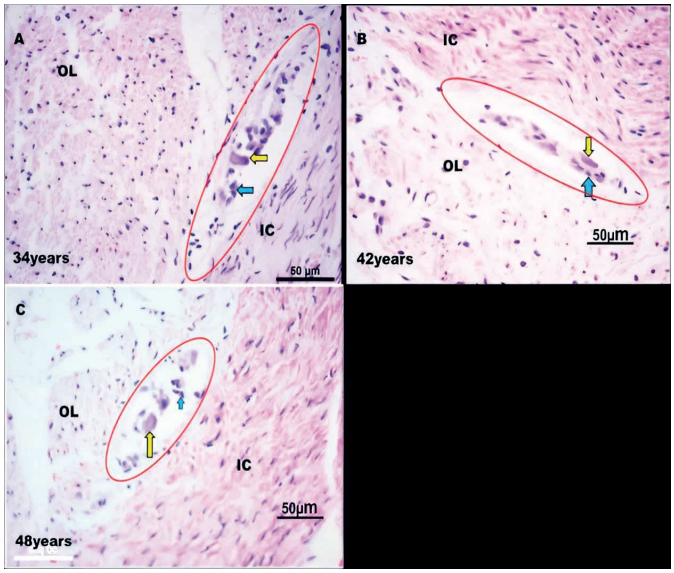

Fig. 2 Photomicrographs (40×) of Hematoxylin and Eosin-stained transverse sections from individuals aged 34 years (A), 42 years (B), and 48 years (C). Myenteric ganglia (MG) (red circle) and their organization within the muscle layers are shown. Myenteric neurons (yellow arrows) and EGCs (cyan arrows) are labelled. Scale bar: 50 µm.

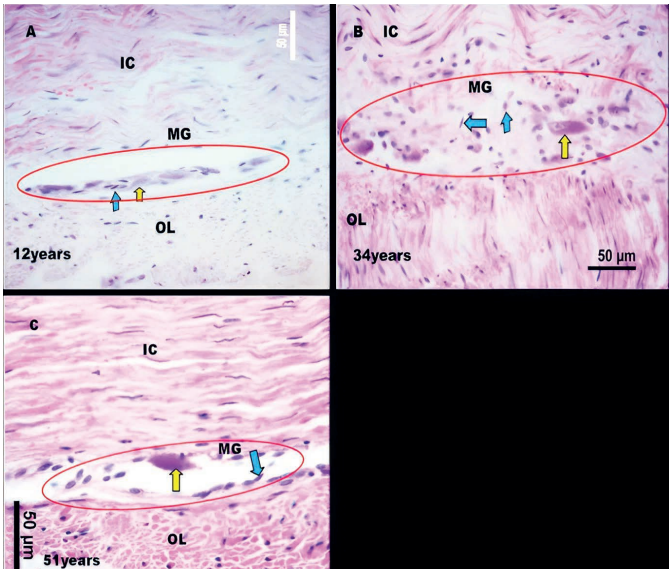

Fig. 3 Photomicrographs (40×) of Hematoxylin and Eosin-stained transverse sections from individuals aged 12 years (A), 34 years (B), and 51 years (C). Myenteric neurons (yellow arrows) and EGCs (cyan arrows) are labelled to illustrate their distribution within the myenteric ganglia (MG). Scale bar: 50 µm.

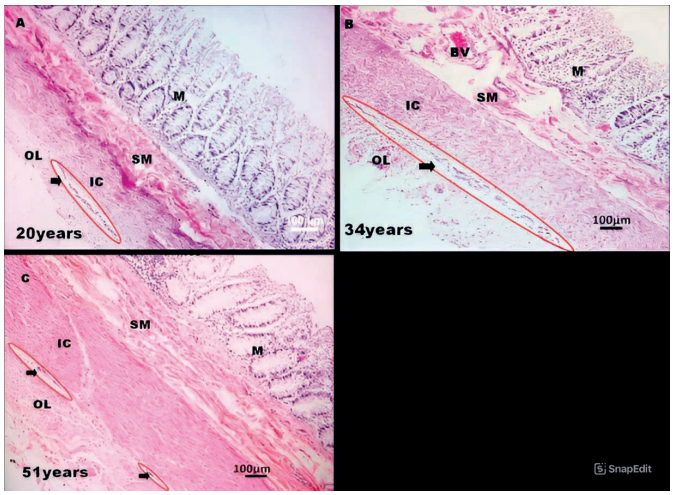

Fig. 4 Photomicrographs (10×) of Hematoxylin and Eosin-stained sections from individuals aged 20 years (A), 34 years (B), and 51 years (C). Myenteric ganglia (MG) (black arrowheads) are shown in relation to surrounding layers including mucosa (M), submucosa (SM), inner circular (IC), and outer longitudinal (OL) muscle layers. Scale bar: 100 µm.

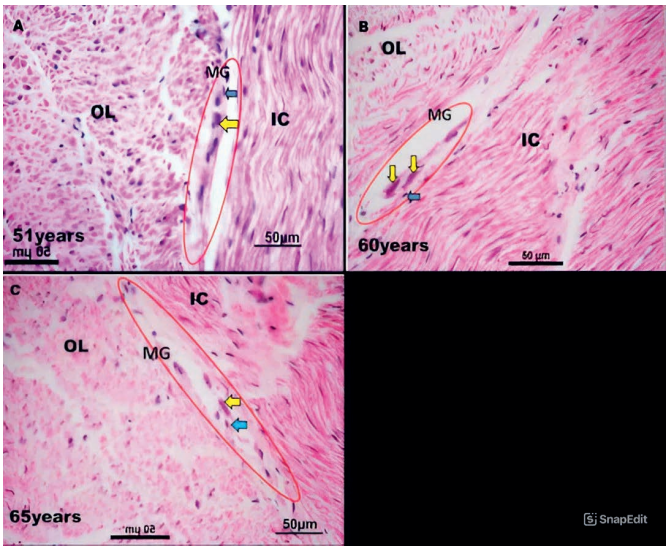

Fig. 5 Photomicrographs (40×) of Hematoxylin and Eosin-stained sections from individuals aged 51 years (A), 60 years (B), and 65 years (C). Myenteric ganglia (MG), myenteric neurons (yellow arrows), and EGCs (cyan arrows) are labelled. Scale bar: 50 µm.

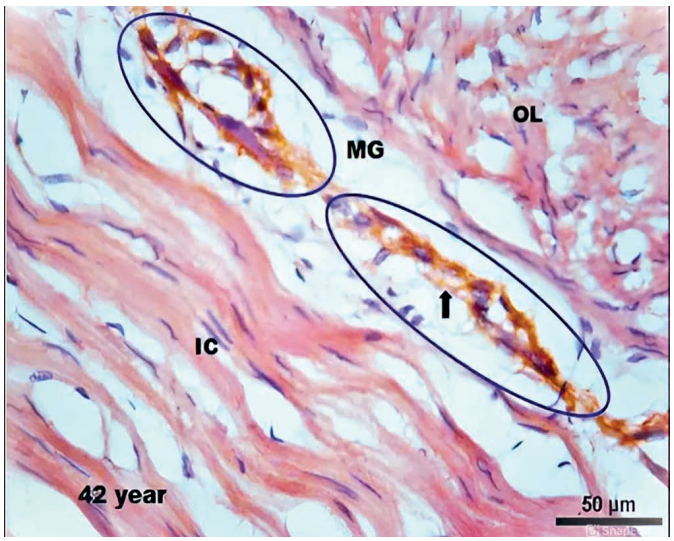

Fig. 6 Photomicrograph (40×) of GFAP-immunostained transverse section from a 42-year-old. Enteric glial cells (EGCs) and their processes (arrowheads) are visualized within myenteric ganglia (MG). IC – inner circular muscle; OL – outer longitudinal muscle. Scale bar: 50 µm.

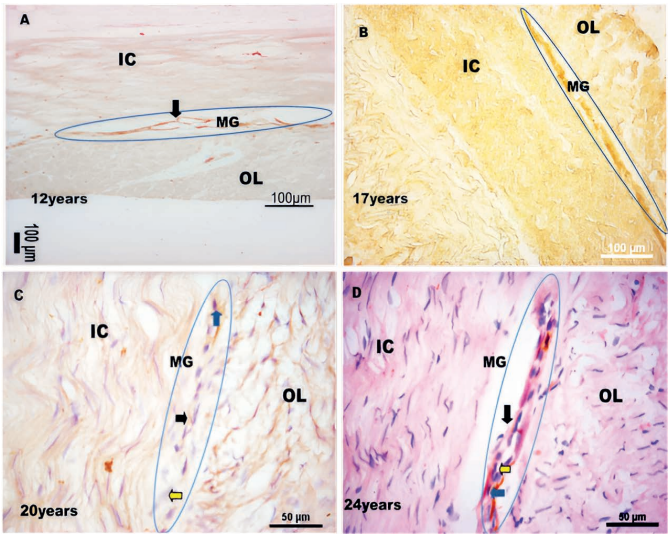

Fig. 7 Photomicrographs of GFAP-immunostained transverse sections from individuals aged 12 years (A), 17 years (B), 20 years (C), and 24 years (D). Neurons (yellow arrows), EGCs (cyan arrows), and their processes (black arrowheads) are shown in myenteric ganglia (MG). Scale bars: 100 µm (A, B) – 20×; 50 µm (C, D) – 40×. IC – inner circular muscle; OL – outer longitudinal muscle.

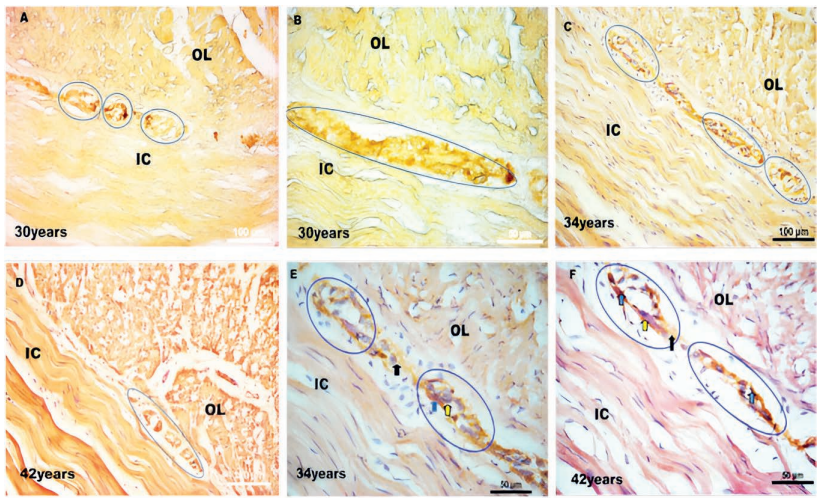

Fig. 8 Photomicrographs of GFAP-immunostained transverse sections from individuals aged 20 years (A), 30 years (B), 34 years (C), 42 years (D), 48 years (E), and 51 years (F). Myenteric ganglia (MG), neurons (yellow arrows), EGCs (cyan arrows), and EGC processes (black arrowheads) are labelled. Scale bars: 100 µm (A) – 20×; 100 µm (C, D) – 10×; 50 µm (B, E, F) – 40×.

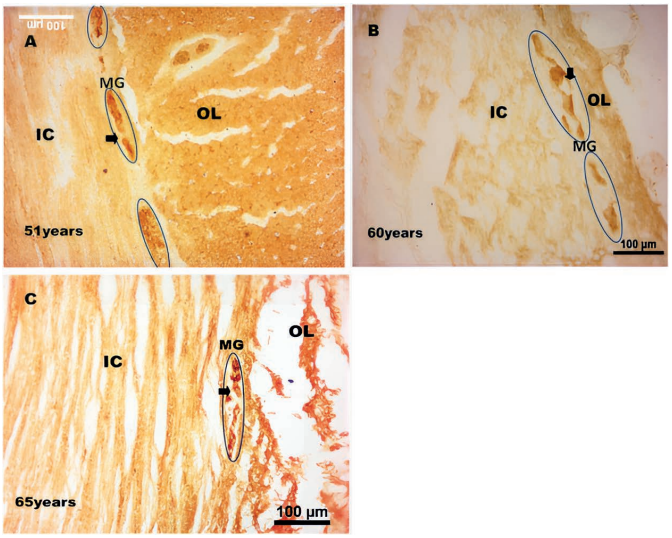

Fig. 9 Photomicrographs (20×) of GFAP-immunostained transverse sections from individuals aged 51 years (A), 60 years (B), and 65 years (C). Myenteric ganglia (blue ovals) and EGC processes (black arrowheads) are labelled. Scale bar: 100 µm.

MORPHOMETRIC EVALUATION

For quantitative analysis, the samples were divided into two groups:

Group 1 Young age (12–34 years, n = 6) and Group 2 Middle-aged (42–65 years, and n = 5).

There was a decrease in GFAP-positive EGCs (both in expression of counts per ganglionic area and per ganglion) in the transverse colon of the middle-aged group. The difference in the count of EGCs per ganglion and the mean count of EGCs per mm² of ganglionic area was significant (p =< 0.05). The mean count of EGCs per MG was 12.5 ± 1.9 and 8.2 ± 1.7 in the young age and middle-aged group, respectively (p = 0.002) (Figure 10). The mean count of EGCs per mm² of ganglionic area was 7.5 ± 1.6 and 1.4 ± 0.2 in the young age and middle-aged group, respectively. (p =< 0.0001) (Figure 11).

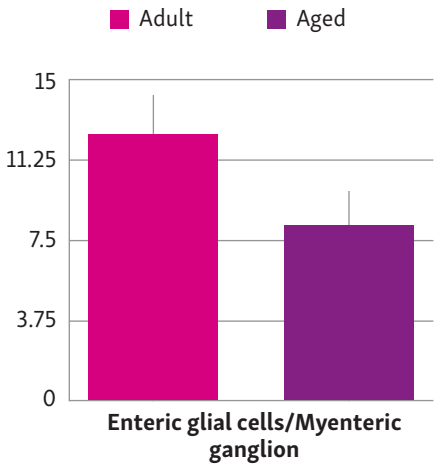

Fig. 10 Graph showing the mean number of GFAP-positive enteric glial cells (EGCs) per myenteric ganglion in **Group 1 (Adults, 12–34 years, n = 6)** and **Group 2 (Middle-aged, 42–65 years, n = 5)**. The mean EGC count per ganglion was **12.5 ± 1.9** in Group 1 and **8.2 ± 1.7** in Group 2, demonstrating a significant decrease in the middle-aged group (**p = 0.002**).
Data are presented as **mean ± SD**.

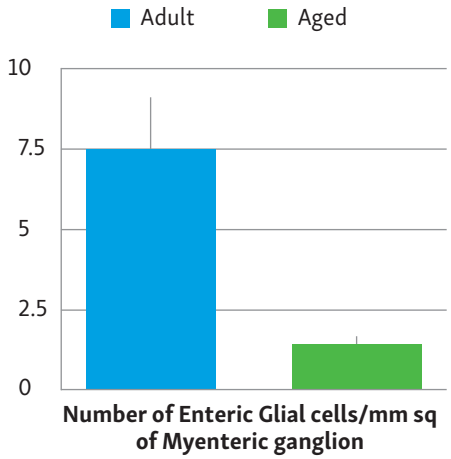

Fig. 11 Graph showing the mean density of GFAP-positive enteric glial cells (EGCs) per mm² of myenteric ganglionic area in **Group 1 (Young, n = 6)** and **Group 2 (Middle-aged, n = 5)**. The mean EGC density was **7.5 ± 1.6 cells/mm²** in Group 1 and **1.4 ± 0.2 cells/mm²** in Group 2. This difference was statistically significant (**p < 0.0001**). Data are shown as **mean ± SD**.

The myenteric fraction per inner circular muscle was significantly greater that is 0.4 ± 0.1 in group 1 Young age (<40yrs) and it was 0.2 ± 0.11 in group 2, Middle-aged (≥40yrs) (p = 0.01) (Figure 12). The number of EGCs increases with age from 12–34 yr and reduces with middle age onwards that is 42 yrs onwards (Figure 13).

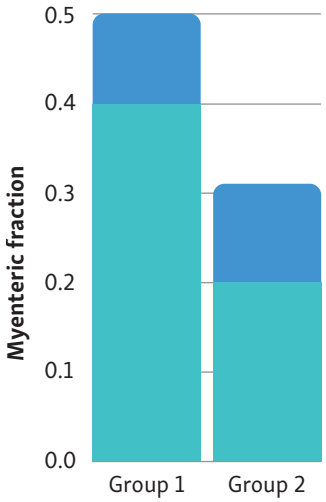

Fig. 12 Graph showing the myenteric fraction per inner circular muscle layer in **Group 1 (Young <40 years, n = 6)** and **Group 2 (Middle-aged ≥40 years, n = 5)**. The myenteric fraction was significantly greater in Group 1 (**0.4 ± 0.1**) compared with Group 2 (**0.2 ± 0.11**), with **p = 0.01**. Values are presented as **mean ± SD**.

DISCUSSION

Age-related changes in EGCs in the human colon are of significant interest to researchers globally. Although numerous studies have been conducted on animal models, there is limited and less significant data available for human specimens. To the best of our knowledge, this is the first study examining the morphological changes of EGCs in the human transverse colon (TC) within the Indian population. The aim of this study is to investigate whether

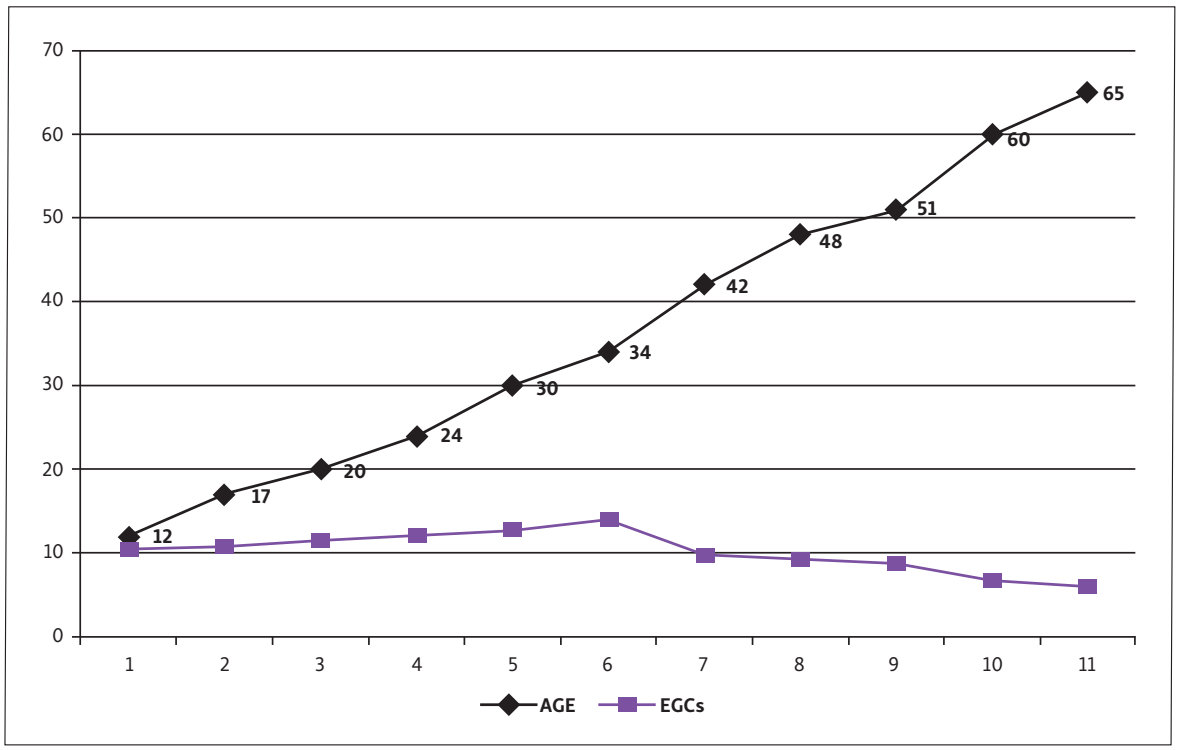

Fig. 13 Graph showing the distribution of GFAP-positive enteric glial cells (EGCs) across the full age range (12–65 years; total n = 11). Each point represents an individual measurement. The data demonstrate an **increase in EGC counts from 12 to 34 years**, followed by a **reduction beginning at 42 years and continuing through 65 years**.

EGCs in the human transverse colon undergo morphological changes with age.

Morphological changes in the MG of the human TC at various ages were observed in the present study. Similar to findings in old guinea pigs, which reported separation of MG and less densely packed myenteric neurons (27), the current study also observed that MG appeared separated from 42 years onwards, with maximum spacing at 65 years of age. Neurons were found to be less densely packed starting at 42 years. Additionally, a reduction in the size of MG was noted in middle-aged individuals, which was more evident in whole-mount preparations and H&E staining. An increase in the myenteric fraction was observed from 12 to 34 years, while a decrease was noted from 42 to 65 years. These observations are consistent with previous studies, which also reported a reduction in the size of MG with age in the ileum of guinea pigs (28). The observed gaps and spacing within MGs in the human colon are attributed to stretching effects from gut growth, with changes in MG shape occurring due to peristalsis and gut movements (29).

This study examined age-related morphological changes in myenteric neurons and EGCs of the human TC, noting alterations in size, shape, and distribution. Previous research has documented age-related loss of enteric neurons in the human esophagus, with evidence of reduced peristaltic contractions in the lower esophagus of the middle-aged (30). The population of enteric neurons in humans begins to decline at an early age, with decreases in submucosal and myenteric plexuses reported as early as the fourth year (31). In this study, a significant reduction in the number of myenteric neurons was observed in middle-aged individuals 40 years and older (Group 2) compared to those under 40 years (Group 1). A reduction in myenteric neurons was noted from 42 years onwards. Previous research has also reported a loss of cholinergic neurons in ageing rats (32). Additionally, the number of neurons can be influenced by diet, microbiota, and calorie intake. Gut growth may cause apparent reductions in neuron density due to a dilution effect. Furthermore, reduced motility can lead to fewer bowel movements and subsequent loss of myenteric neurons. Observed spaces within and between myenteric ganglia support the idea of neuron loss (33). Hippocrates observed that intestinal function slows with age. Contemporary research shows that age-related changes in the ENS can influence GI function. Conditions such as diarrhea and fecal incontinence (34), irritable bowel syndrome (35), and gastroesophageal reflux disease (36) are commonly seen in middle-aged and older adults and can significantly impact their quality of life and independence.

In the present study, myenteric neurons were found to be smaller in individuals under 42 years of age and increased in size with age, with larger neurons observed in those above 42 years. This increase in neuron size with age is consistent with observations in older rats (32). The shape of myenteric neurons in the present study varied, with neurons appearing irregular, spherical, or oval, and some exhibiting a horny profile. Similar horny profiles have been noted in myenteric neurons of ageing animals (27). In middle-aged individuals (above 42 years), some large myenteric neurons with smooth profiles were observed at the edges of MG, and some extra ganglionic neurons were noted within the myenteric plexus. Previous studies have also reported peripherally located myenteric

neurons in humans and extra-ganglionic neurons in the ileum of ageing guinea pigs (37).

Human EGCs in the ENS express GFAP, a protein associated with glial intermediate filaments (38). This study observed a decline in EGCs starting from age 42, correlating with a loss of myenteric neurons. Previous research on rats has shown significant EGC loss in various intestinal regions at ages 5–6 months and 26 months, except the rectum (39). This age-related reduction may indicate deficits in neural stem cells or mechanisms regulating their differentiation into glial cells, potentially due to increased apoptosis. In this study, EGC loss was notably greater in individuals aged 40 years and older. Additionally, EGCs, which have numerous processes, were observed to decrease with age, consistent with findings from previous studies (37). Previous research has shown that Sox-10-, S100-, and GFAP-IR EGC expression is present in the human colon and varies with age and colonic location. Therefore, in the myenteric and CM of the old person, there is a decrease in the density of S100-IR EGCs, but this reduction is not followed by a loss of Sox10-IR EGCs in the MP. The absence of GFAP expression in the middle-aged samples would suggest that EGCs do not become activated as people age. Findings from prior work in the human descending colon demonstrate that ageing induces selective alterations in enteric glial subpopulations rather than a uniform loss of glial cells. Sox10-positive enteric glia, representing the total glial pool, were largely preserved in both myenteric and submucosal plexuses of older individuals, indicating that chronological age alone does not cause widespread depletion of glial cell bodies. In contrast, S100-positive glia showed a marked reduction in density, particularly within the myenteric plexus and circular muscle, suggesting a specific vulnerability or phenotypic shift of this subset with advancing age. These authors also reported region-dependent changes in additional markers, such as GFAP, consistent with a remodelling of glial phenotype and distribution across the colonic wall. Notably, the absence of GFAP expression in the middle-aged group in our study further suggests that enteric glia may not undergo GFAP-associated activation during normal ageing. Together, these observations support the presence of significant heterogeneity within human EGC populations, reflected in the variable expression of Sox10, S100, and GFAP, and indicate that while the fundamental glial scaffold is maintained, ageing drives marker-specific and region-specific adaptations that may alter glial support for neurons, smooth muscle, and overall gut homeostasis (40).

Immunocytochemical studies have revealed that neurodegeneration and a decline in EGCs begin after age 40. This loss is significant because it is associated with various GI disorders, including fecal incontinence and constipation, which heavily impact quality of life and healthcare costs in the middle-aged. The reduction in EGCs with age might suggest a deficit in neural stem cells or issues with their differentiation into EGCs, potentially due to increased apoptotic activity. However, the study has several limitations: it involved a small sample size per age group, lacked information on dietary conditions of the deceased, and did not differentiate between types of EGCs. Additionally, the study did not explore sex-related differences between males and females. Using whole-mount preparations (41) might have offered more detailed insights. Furthermore, the absence of a pan-EGC biomarker remains a significant challenge in ageing research. We acknowledge that the interpretation of glial cell shape is limited by the use of paraffin-embedded transverse sections, as sectioning angle can influence apparent morphology. Lastly, inclusion of individuals <18 years may introduce developmental variability and represents a limitation of the study.

CONCLUSION

This study outlines age-related morphological variations of enteric glial cells (EGCs) in the myenteric plexus of the human colon, influenced by gut growth and responses to dietary and environmental changes. The number of myenteric neurons and EGCs appeared to decrease with increasing age. The decreased GFAP expression and morphometric changes observed in middle-aged samples (Group 2) may suggest that enteric glial cells are less activated or functionally impaired with advanced age. These findings are valuable for clinicians, surgeons, and scientists in enhancing the understanding of functional bowel disorders in geriatric age groups.

AUTHOR CONTRIBUTIONS

AA and SS[1] contributed substantially to the conception and design of the study. AA, AV and SR contributed substantially to the acquisition of data. AA, VD, AV, SR, SS[2] and SS[1] made substantial contributions to the analysis and interpretation of data. AA and SS[1] drafted the manuscript. AA, VD AV, SR, SS[2] and SS[1] critically revised the manuscript for important intellectual content. All the authors approved the final version submitted for publication and took responsibility for statements made in the published article.

CONFLICTS OF INTEREST STATEMENT

None of the authors has any potential or actual conflicts of interest concerning the published article to disclose.

DATA SHARING STATEMENT

All data generated or analyzed during the present study are included in this published article.

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