



## Original Research Article

## Correlation of number of mast cells and thickness of submucosal nerve fibers in mesenteric and antimesenteric halves of the doughnut biopsy in hirschsprung's disease

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## ABSTRACT

**Introduction:** Hirschsprung's disease (HSD) is a congenital malformation of the hindgut characterised by absence of ganglion cells. The mast cells (MC) population is low in ganglionic segment of the intestine when compared to the aganglionic segment. The conclusive role of these cells is yet to be proven.

**Objectives:** To quantify the mast cells in mesenteric and antimesenteric halves of the colonic doughnut biopsy in cases of HSD. Evaluation of mast cell distribution in relation to thickness of the nerve fibers in doughnut biopsies in cases of HSD.

**Materials and Methods:** This study was conducted for one year in Bangalore medical college and research institute, in the department of pathology from November 2017 to November 2018. 16 levels of doughnut biopsies from 10 patients with confirmed HSD were received. Toluidine blue stain was done to evaluate for the MC distribution in the mesenteric and antimesenteric halves.

**Results:** In our study when the number of MC were correlated with the thickness of the largest nerve fiber, it was observed that maximum correlation was seen in the anti-mesenteric half (correlation coefficient = 0.70) when compared to only some correlation seen in the mesenteric half (correlation coefficient 0.39).

**Conclusion:** MC are increased in number with increase in thickness of the nerve fibers in the submucosa of doughnut biopsies. There was no difference in the distribution of MC between the mesenteric and antimesenteric halves of doughnut biopsy.

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### 1. Introduction

Hirschsprung's disease (HSD) is a congenital malformation of the hindgut characterised by absence of intrinsic ganglion cells in the submucosal and myenteric plexus. It occurs in approximately 1 in 5000 live births.<sup>1,2</sup> There has been a lot of interest in the role of mast cells (MC) in the pathogenesis of HSD.

MC derived from bone marrow precursor cells, are widely distributed in mucous membranes and connective tissues of the respiratory, gastrointestinal, and urinary tracts, and are important effector, regulatory immune cells which play a key role in the nerve-immune-endocrine network.<sup>3</sup> It is observed that the mast cells (MC) population is low in

ganglionic segment of the intestine when compared to the aganglionic segment.

Ganglionation of the intestine in HSD is not a uniform process and transition zone is the growing front. Non-affected segment of the intestine to be labeled as functional, should not only show adequate ganglionation but there should always be absence of hypertrophic nerve fibers in the submucosa. It is not necessary that the entire circumference of the doughnut biopsy needs to be ganglionated, especially when sampled from the transition zone. The reason why it is necessary to sample all the 4 quadrants of given doughnut biopsy.

Previous studies have evaluated for distribution of MC in only the aganglionated and ganglionated segments of HSD cases. None of the studies so far have tried to evaluate and

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quantify the presence of mast cells in the transition zone with reference to mesenteric and antimesenteric halves.

Since the mast cells are usually seen accompanying the neurovascular bundle and mast cells via their mediators may affect the nerve fibers, this is an indirect effort to quantify and correlate the presence of MC in relation with the nerve fibers.

## 2. Materials and Methods

16 levels of doughnut biopsies from 10 patients of confirmed HSD were received in the department of pathology BMCRI during the study period.

Formalin fixed paraffin embedded sections stained with Hematoxylin and Eosin were screened for the presence or absence of ganglion cells in all the 4 quadrants of myenteric plexus and the thickness of the largest nerve fiber present, if any in the submucosa of both mesenteric and antimesenteric halves of the doughnut biopsy were noted down.

Toluidine blue staining on paraffin sections was performed using 1% aqueous solution of toluidine blue for 20 min. Mast cells were counted around the thickest nerve fiber present in five successive high power fields ( $\times 400$ ) in the submucosa.<sup>4</sup>

## 3. Results

Out of the 16 levels of doughnut biopsies from 10 patients with confirmed HSD, 2 of the doughnut biopsies did not show any evidence of nerve fibers in the sub mucosa and were excluded from the study. Rest of the 14 levels of doughnut biopsies from 8 patients were analysed in this study.

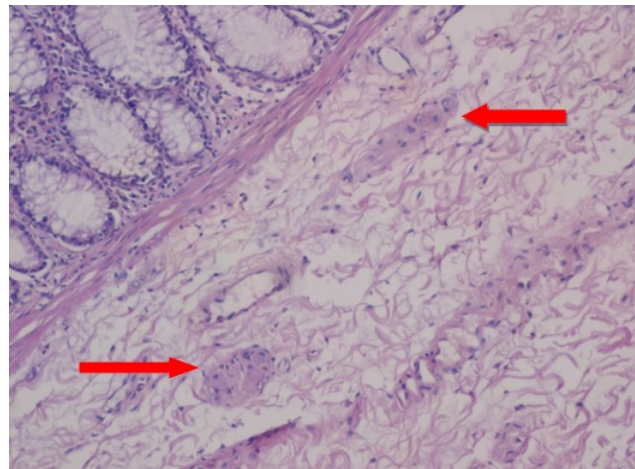
### 3.1. Mast cells

The data obtained after analysing the number of mast cells in both mesenteric and antimesenteric halves of the doughnut biopsies showed that the number of mast cells in mesenteric half was mean  $\pm$  2SD = 20.4  $\pm$  13.6. Number of mast cells in anti-mesenteric half was mean  $\pm$  2SD = 20.7  $\pm$  15.7. The difference between the two was not statistically significant ( $p=0.46$ ).

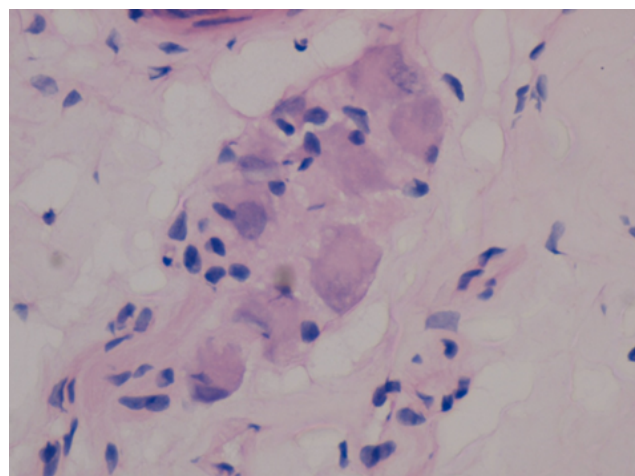
### 3.2. Mast cells and nerve fibers

In our study when the number of mast cells were correlated with the thickness of the largest nerve fiber, it was observed that maximum correlation was seen in the anti-mesenteric half (correlation coefficient = 0.70) when compared to only some correlation seen in the mesenteric half (correlation coefficient = 0.39).

That is there was increase in the number of MCs with increasing thickness of the nerve fiber in the submucosa which was strikingly significant in the anti-mesenteric half of the doughnut biopsy.



**Fig. 1:** H and E stain of doughnut biopsy showing mucosa and submucosa with submucosa showing ganglion cells (arrows). 10x



**Fig. 2:** H and E stain of doughnut biopsy showing clusters of ganglion cells in the submucosa. 40x

## 4. Discussion

Previous studies have focused on MC distribution in ganglionic and aganglionic segment of intestine. Our study was aimed at evaluating distribution of MC in entire circumference of doughnut biopsy.

The mast cells were distributed around the nerves and blood vessels in addition to being randomly scattered. There was no significant difference seen in the distribution of these MCs in both mesenteric and anti-mesenteric halves of the doughnut biopsy ( $p=0.46$ ).

We also correlated thickness of the nerve fibers obtained by image analysis morphometry with MC number. It was observed that maximum correlation was seen with size of nerve fibers in the antimesenteric halves (correlation coefficient = 0.70) and some correlation was seen in mesenteric halves (correlation coefficient = 0.39). This is

**Table 1:** Thickness of the nerve fibers and number of mast cells in mesenteric half of the doughnut biopsy.

S. No	Mesenteric half		
	Ganglion cells	Thickness of nerve fiber (um)	No. of mast cells
1	P	28.4	51
2	P	32.7	38
3	P	25.2	20
4	P	30.1	7
5	P	20.9	38
6	P	11.5	8
7	P	24.3	21
8	P	16.7	12
9	P	29.3	21
10	P	21.3	19
11	P	31.8	25
12	P	25.3	12
13	P	9.6	8
14	P	28.8	6

P= Present

**Table 2:** Thickness of the nerve fibers and number of mast cells in anti-mesenteric half of the doughnut biopsy.

S. No	Anti-mesenteric half		
	Ganglion cells	Thickness of nerve fiber (um)	No. of mast cells
1	P	27.1	22
2	P	44.2	66
3	P	14.8	10
4	P	17.3	6
5	P	15.8	18
6	P	10.2	6
7	P	15.7	9
8	P	30.2	27
9	P	16.4	33
10	P	27.6	30
11	P	24.5	23
12	P	29.1	17
13	P	29.4	17
14	P	27.1	22

P= Present

**Table 3:** Mean mast cell numbers found in different studies compared to the present study.

	Our study	Kobayashi et al <sup>5</sup>	Demirbilek et al <sup>6</sup>	Yadav AK et al <sup>4</sup>	Shiqi Wang et al <sup>3</sup>	Hermanowicz A et al <sup>7</sup>
Mesenteric half	20.4±13.6	23.9±6.6	18.2±3.3	36.36±39.58	19.64±3.62	121.32±24.21
Anti-mesenteric half	20.7±15.7					

in contrast to the findings of Yadav AK et al where they observed that there was maximum correlation of MC with the number of nerve fibers in cases of HSD.

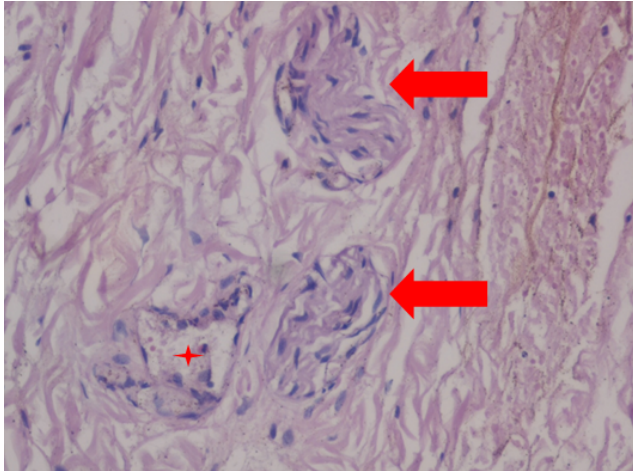
Mast cells are known to secrete substances like nerve growth factor which may influence the thickness of the nerve fibers. Additionally, MC products modulate inflammation processes thus influencing on the clinical course of HSD.

In view of strong correlation between the number of mast cells with increasing thickness of the nerve fiber in the doughnut biopsies in cases of HD, we suggest that in

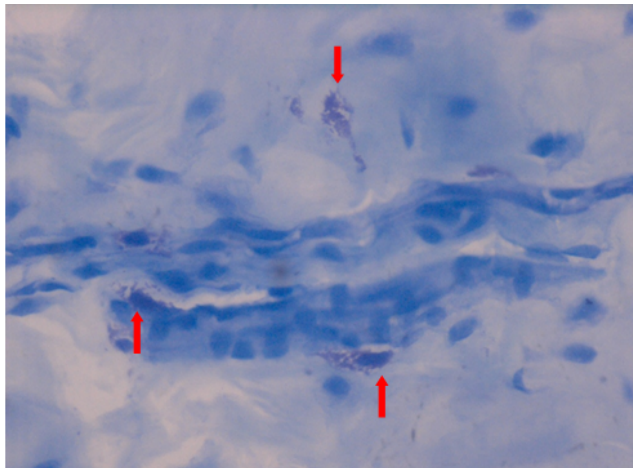
the evaluation of presence or absence of nerve fibers in submucosa of a doughnut biopsy the quantification of these mast cells may provide an indirect clue that there may be presence of thickened nerve fiber. Detection of which helps in identifying the transition zone.

## 5. Conclusion

MCs are increased in number with increase in thickness of the nerve fibers in the submucosa of doughnut biopsies. There is no difference in the distribution of MCs between



**Fig. 3:** H and E stain of doughnut biopsy showing two nerve fibers (arrows) in close proximity of a vessel (star) within submucosa. 40x



**Fig. 4:** Toluidine blue stain of doughnut biopsy showing mast cells (arrows) in close proximity with two nerve fibers within submucosa. 40x

the mesenteric and antimesenteric halves of doughnut biopsy.

Quantification of MCs in doughnut biopsies gives a clue regarding presence or absence of thickened nerve fibers in the submucosa in case of non-availability of morphometry, which helps to identify the transition zone.

## 6. Source of funding

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## 7. Conflict of interest

None.

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