# Increased mast cell density in haemorrhoid venous blood vessels suggests a role in pathogenesis

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## ABSTRACT

**Introduction: Haemorrhoids are an abnormal, tortuous dilatation of the arteriovenous plexus of the anus. Although increased resting anorectal pressure is deemed to be a major initiating factor, a thorough understanding of the pathogenesis is still lacking. Mast cells, through release of granules, can affect local vessels with respect to changes in calibre, changes in permeability and thrombosis. Thus, mast cells could play a role in haemorrhoid pathophysiology, although this has not been previously investigated.**

**Methods: 48 cases of haemorrhoids were retrospectively collected at King Chulalongkorn Memorial Hospital, with normal anorectal tissue from surgically-removed colorectal cancer serving as controls. Mast cells were identified by toluidine blue staining and quantitated around venous vessels.**

**Results: Mast cells around haemorrhoidal vessels were significantly more numerous than in normal specimens (p-value is less than 0.001). Similar values were found for haemorrhoids showing chronic changes and those in a more acute stage.**

**Conclusion: These findings support the hypothesis that mast cells may play a role in the pathophysiology of haemorrhoids. Mast cells appear to participate equally in the early and later stages of these lesions. Mast cells are known to affect local vascular conditions through release of their chemical mediators and cytokines, and may influence haemorrhoid symptomatology and progression at this level.**


**Keywords: anorectal tissue, cytokines, haemorrhoids, haemorrhoid pathophysiology, inflammatory mediators, mast cells**

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## INTRODUCTION

Haemorrhoids are a common anorectal problem characterised by an abnormally distended submucosal arteriovenous shunt, embedded in swollen and inflamed anal cushions. The arteriovenous plexus is formed between the terminal branches of the superior rectal artery and the superior, middle and inferior rectal veins.[1,2] Common symptoms of external haemorrhoids (found below the dentate line) are bleeding and pain from vascular thrombosis, whereas internal haemorrhoids (above the dentate line) protrude into the lumen, leading to rectal mucus deposition on perianal skin that can lead to irritation and discomfort.[3] The exact pathogenesis of this condition is still poorly understood. Although most physicians believe that prolonged straining to move the bowels plays a major aetiologic role, other factors, such as changes in bowel habit, pregnancy, low fibre intake and family history, also contribute to the development of haemorrhoids [4,5]

Haemorrhoids can be thought of as a localised vascular disturbance, and some of the changes observed in these lesions are processes that are known to be influenced by mast cells in other sites. When mast cells reach target tissues, they release various mediators from their granules.[6] Biogenic amines, in particular histamine and leukotrienes, induce vasoconstriction and vascular permeability.[7] Enzymes, consisting mainly of tryptase and chymase, can promote vascular breakdown or vessel wall weakness, leading to tortuosity and/or neovascularisation.[6] Platelet-activating factor enhances thrombocytic aggregation and vasodilatation.[8] All these vascular effects can be identified in haemorrhoids. We therefore postulated that mast cells may play a role in the pathogenesis of this condition. If so, one might expect increased numbers of mast cells in haemorrhoids. As no such data is available in the literature, we carried out this study to quantitate mast cells in haemorrhoids.

## METHODS

48 cases diagnosed to have haemorrhoids were retrieved from the pathology files at King Chulalongkorn Memorial Hospital between the years 2001 and 2005. None of

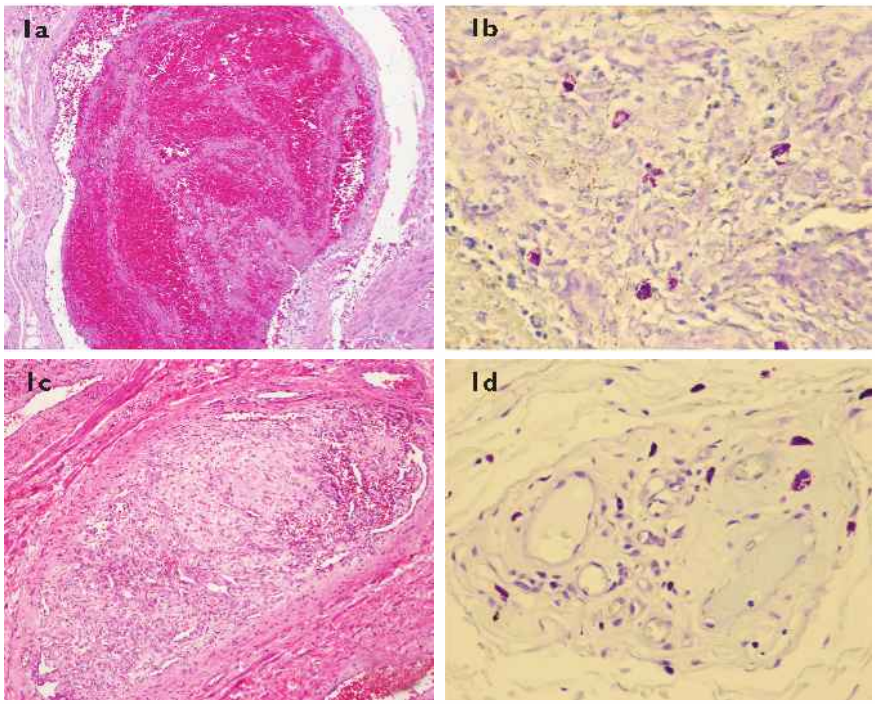


**Fig. 1** Photomicrographs show mast cells in the acute and chronic stages of haemorrhoids. (a) Acute haemorrhoid with thrombus composed of packed red blood cells and fibrin materials (Haematoxylin and eosin, × 200). (b) Mast cells with intracytoplasmic metachromatic granules within an area of marked inflammation in an acute haemorrhoid (Toluidine blue, × 600). (c) Chronic haemorrhoid with a vein showing occlusion by proliferating fibrous tissue and recanalisation (Haematoxylin and eosin, × 200). (d) Mast cells around recanalised capillaries in a chronic haemorrhoid (toluidine blue, × 600).

the patients had other coexisting bowel disorders. Following review, each case was classified as chronic if haemorrhoidal veins contained proliferative fibrous tissue or showed remote occlusion with recanalisation. Otherwise, lesions were classified as acute. Mast cell density was assessed on 5 micron sections, stained with 0.1% aqueous toluidine blue. Mast cells were identified as cells with intracytoplasmic metachromatic granules. For each case, the four areas of highest mast cell density were selected by microscopic examination of the slides and then photographed under a Nikon Eclipse E600W microscope equipped with a digital Nikon camera and DXM1200F software. Mast cells around vessels were counted manually in each of the four fields, and results were combined to arrive at a count for mast cells/mm$^2$. For controls, tissue blocks from the distal anorectal margins of surgically-removed colorectal cancer specimens were used. A total of 48 cases were studied. Slides were reviewed to confirm that no pathology was present. The mean mast cell densities between haemorrhoid cases and controls, and acute versus chronic cases, were compared by Student *t*-test, using the Statistical Package for Social Sciences version 11.5 (SPSS Inc, Chicago, IL, USA). Confidence intervals were at 95%. A p-value of $< 0.05$ was considered to be statistically significant.

## RESULTS

There were 22 cases of internal, and 26 cases of external haemorrhoids for analysis, with the mean (and standard deviation [SD]) age of 54 (± 14.0) years. 28 of the cases were classified as acute, and the other 20 cases were classified as chronic (Fig. 1). The control group consisted of 48 cases, with mean (SD) age of 61 (± 13.8) years. The mean (SD) values for mast cell density around venous blood vessels in haemorrhoids and controls were 64 (± 20.9) and 24 (± 12.9) cells/mm$^2$, respectively. This difference was statistically significant ($p < 0.01$). The mean (SD) values of mast cell density in acute and chronic haemorrhoids were 62 (± 19.6) and 65 (± 24.0) cells/mm$^2$, respectively (Fig. 1). The difference between these groups was not statistically significant ($p = 0.3$).

## DISCUSSION

Mast cell populations are known to be significantly increased in certain physiological and pathological conditions, such as wound healing, rheumatoid arthritis, idiopathic pulmonary fibrosis, cutaneous haemangioma, and various solid malignancies.[9,10] Many of these conditions involve angiogenesis and/or fibrosis, processes that are enhanced by mast cells.[10] Several mediators and enzymes of their granules promote new

blood vessel formation along with connective tissue degradation to produce spaces for neovascular sprouts. Mast cells are a major source of basic fibroblast growth factor, an important polypeptide for fibrogenesis. In turn, fibroblasts also recruit mast cells in developing fibrous tissue.[10,11] There is a well-recognised relationship between increased numbers of mast cells and proliferative or cellular haemangiomas.[12,13] In one study, mast cell numbers were highest in involuting haemangiomas rather than proliferating ones, suggesting these cells may play a greater role in fibrosis and resolution of these lesions.[13] Non-neoplastic vascular lesions have received little attention. Reports have determined the mast cell density in vascular malformations and varicose veins of lower extremities, but no significant correlations were made.[8,13]

We believe that this is the first such study on haemorrhoidal vessels. We found that there is a significant increase in mast cells in association with haemorrhoids compared to normal tissue from the same site. In contrast to the studies on haemangiomas, we found that there was no difference between mast cell numbers in haemorrhoids showing more chronic changes, and those from more acute stages. This leads us to conclude that mast cells are equally involved in the early and later stages of haemorrhoids.

We propose that mast cells have a multidimensional role in the pathogenesis of haemorrhoids, through the actions of the chemical mediators and cytokines released from mast cell granules. The roles are likely to vary for the early and later stages in the evolution of this lesion. With respect to the early stages, vasoconstriction together with increased vasopermeability and smooth muscle contraction can be induced by histamine and leukotriene from mast cell granules.[6,7] When the distended submucosal veins with weakened vessel walls in haemorrhoids are subjected to these influences, this could promote extravasation of red blood cells and haemorrhage. Mast cells also release platelet-activating factors causing platelet aggregation and thrombosis, which are common acute complications of haemorrhoids.[8,14] In the later stages of the lesion, thrombosed haemorrhoids undergo eventual recanalisation and resolution. This process would be promoted by mast cell granule contents including tryptase and chymase for stromal tissue degradation, heparin for endothelial cell migration, and cytokines such as tumour necrotic factor-$\alpha$ and interleukin 4 for fibroblast growth and proliferation. Moreover, fibrous tissue formation would be promoted by basic fibroblast growth factor from mast cells.[10,15]

It remains to be proven to what extent mast cells actually influence these events in haemorrhoid formation and progression, but we believe this is a concept that deserves further attention. What our study has shown is that the numbers of mast cells in haemorrhoids are abnormally high and such cells are ideally suited for promoting processes that form part of the spectrum of haemorrhoidal disease. As such specimens are relatively common in most pathology departments, they could serve as easily accessible models for studying aspects of mast cell role function in human tissue and for applying concepts from experimental studies to humans.

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