Distribution of Mast Cell in Acute Appendicitis and Its Role in Histopathological Diagnosis - A Study in Tertiary Care Centre from South India

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ABSTRACT

BACKGROUND

Appendicitis is the most common indication for intra-abdominal surgery, especially in adolescents and young adults. In about 15 – 25 % of appendices removed at surgery because of suspected symptoms, histological features of acute appendicitis are absent. The cause of acute abdominal pain in these patients can be due to mast cells which may play a role in pathogenesis of appendicitis-like pain. Demonstration of mast cell in tissue can be done by toluidine blue staining or immunohistochemistry (IHC) marker CD 117. We wanted to evaluate whether the degree of distribution of mast cells has any relation with clinical findings and evaluate its usefulness as a diagnostic marker for histological diagnosis of acute appendicitis.

METHODS

This is a descriptive study done in 120 cases of appendicectomy specimens from clinically diagnosed cases of acute appendicitis received in a tertiary care center in South India in which 60 where histology positive and others were histology negative. Mast cell distribution in each group was compared using toluidine blue stain and CD 117. Collected data was entered in Microsoft excel and analysed using the statistical software SPSS version 16.

RESULTS

Mast cell distribution was significant in all layers of histologically negative acute appendicitis in comparison with histology positive cases. Mucosa has maximum mast cells distribution.

CONCLUSIONS

Mast cells play an important role in the clinical symptoms of patients even when there were no features of acute inflammation. In those cases, mast cells can be demonstrated by simple toluidine blue staining or IHC markers. This is one of the less studied areas, and the clinicopathological discrepancy can be solved by giving mast cell distribution along with histopathology diagnosis.

KEYWORDS

Appendicitis, Histologically Negative Acute Appendicitis, Mast Cells, Toluidine Blue, CD 117

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BACKGROUND

Appendicitis is the most common indication for intra-abdominal surgery, especially in adolescents and young adults.[1] Obstruction and inflammation is implicated in the pathogenesis of acute appendicitis. Histologically acute appendicitis is diagnosed by neutrophilic infiltration in muscularis propria and chronic cases characterised by mononuclear cell infiltrate including lymphocytes and plasma cells.[1] In about 15 – 25 % of appendices removed at surgery because of suspected symptoms, histological features of acute appendicitis are absent.[2] The cause of acute pain in these patients remains unexplained. Mast cells may be playing a role in pathogenesis of appendicitis - like pain and may be leading to fibrosis in chronic cases.[3]

Mast cells are formed in bone marrow and they release many chemical mediators. The most prominent one is histamine which plays a role in the pathophysiology of allergic and autoimmune disease.[4]

Demonstration of mast cell in tissue can be done by most simple toluidine blue staining which is a metachromatic stain. Mast cells give strong membrane and cytoplasmic positivity with IHC marker CD 117. This study evaluates whether the degree of distribution of mast cells has any relation with clinical findings and its usefulness as a diagnostic marker for histological diagnosis of acute appendicitis.

Objectives

1. To study the pattern of mast cell distribution in clinically diagnosed cases of acute appendicitis.
2. To compare the mast cell distribution in histology - positive appendicitis and clinically positive but histologically negative appendicitis.

METHODS

It was a descriptive study done in all appendicectomy specimens from clinically diagnosed cases of acute appendicitis received in a tertiary care center in South India for 6 months from January 2017 to 2017 June. The main objective was to study the pattern of mast cell distribution in clinically diagnosed cases of acute appendicitis and to compare the mast cell distribution in histology-positive appendicitis and clinically positive but histologically negative appendicitis. The study commenced after getting Institutional ethical committee clearance.

Patient details like age, clinical findings, histopathology number were taken from the medical records. The specimens undergo the process of fixation in 10 % neutral buffered formalin.

Cutting, biting, tissue processing and 5 micrometre thickness section stained with H & E slides were analysed under optical microscope. The H & E slides were analysed and categorised as histology-positive acute appendicitis (histology showing neutrophilic infiltration in muscularis propria), clinically acute appendicitis but histologically negative (histology showing no neutrophilic infiltration). Unstained sections of all study specimens were stained with toluidine blue and IHC marker CD117 (c - kit) for mast cells. The distribution of mast cells in mucosa, submucosa, muscularis propria and serosa were counted in a quantitative manner by taking average of mast cells of 10 HPF and mast cell density expressed as mast cells/HPF. The pattern of distribution of mast cells in both the categories was compared. Data was entered in Microsoft Excel and was analysed using the statistical software SSPS version 16.0. For comparison we used Mann-Whitney U Test, Kruskal Wallis Test and significance level calculated by chi-square test.

Inclusion Criteria

All specimens clinically diagnosed as acute appendicitis received in a tertiary care center in South India in 6 months from January 2017 to June 2017.

Exclusion Criteria

a) Histopathology showing gangrene, necrosis
b) Appendicitis associated with other pathological conditions of GIT like inflammatory bowel disease, tuberculosis etc.

RESULTS

During the study period, 120 cases were studied of which 60 were histology positive appendicitis. Males were affected more than females in a ratio of 3 : 2. Of the total number of cases. 45.8 % cases belong to the age group 11 - 20. Mast cell distribution in mucosa, submucosa, muscularis propria and serosa in histologically positive acute appendicitis and histologically negative acute appendicitis were significant and comparable with toluidine blue staining and CD117.

By CD 117, the mean distribution of mast cell in mucosa is significantly more in histology negative acute appendicitis (HNAA) compared to histology positive acute appendicitis (HPAA) (P - value of 0.030) (Table 1 - 4)

Mucosa	HPAA		HNAA		Z#	P
	Count	Percent	Count	Percent		
Nil	0	0.0	6	10.0		
1	14	23.3	6	10.0		
2	27	45.0	36	60.0		
3 - 4	10	16.7	2	3.3	0.69	0.490
> = 5	9	15.0	10	16.7		
Mean ± SD	3.2 ± 3.6		3.1 ± 3.6			
Median	2.0		2.0			
Sub mucosa	HPAA		HNAA		Z#	P
	Count	Percent	Count	Percent		
Nil	3	5.0	3	5.0		
1	26	43.3	17	28.3		
2	18	30.0	19	31.7		
3 - 4	2	3.3	11	18.3	1.49	0.137
> = 5	11	18.3	10	16.7		
Mean ± SD	2.3 ± 1.9		2.9 ± 2.5			
Median	2.0		2.0			

Table 1. Comparison of MCD in Mucosa & Submucosa between Type of Appendicitis under Toluidine Blue Staining

Mann - Whitney U Test

Muscularis Propria	HPAA Count	HPAA Percent	HNAA Count	HNAA Percent	Z#	p
Nil	2	3.3	12	20.0		
1	37	61.7	29	48.3		
2	16	26.7	13	21.7		
3 - 4	3	5.0	6	10.0	1.41	0.158
> = 5	2	3.3	0	0.0		
Mean ± SD	1.5 ± 1		1.3 ± 1.1			
Median	1.0		1.0			
Serosa	HPAA Count	HPAA Percent	HNAA Count	HNAA Percent	Z#	p
Nil	2	3.3	12	20.0		
1	44	73.3	30	50.0		
2	12	20.0	15	25.0		
3 - 4	2	3.3	3	5.0	0.67	0.502
Mean ± SD	1.3 ± 0.7		1.2 ± 0.9			
Median	1.0		1.0			

Table 2. Comparison of Muscularis Propria & Serosa with Regard to the Type of Appendicitis under Toluidine Blue Staining

Mann - Whitney U Test

Mucosa	HPAA Count	HPAA Percent	HNAA Count	HNAA Percent	Z#	p
2	2	3.3	10	16.7		
3 - 4	6	10.0	7	11.7		
> = 5	52	86.7	43	71.7	2.17*	0.030
Mean ± SD	8.9 ± 4.7		8.7 ± 5.2			
Median	8.0		8.5			
Sub mucosa	HPAA Count	HPAA Percent	HNAA Count	HNAA Percent	Z#	P
1	16	26.7	13	21.7		
2	12	20.0	20	33.3		
3 - 4	10	16.7	19	31.7	1.28	0.199
> = 5	22	36.7	8	13.3		
Mean ± SD	3.5 ± 2.2		3 ± 2.2			
Median	3.0		2.0			

Table 3. Comparison of Mucosa & Sub Mucosa with Regard to the Type of Appendicitis by CD 117

Mann - Whitney U Test

Muscularis Propria	HPAA Count	HPAA Percent	HNAA Count	HNAA Percent	Z#	p
1	28	46.7	32	53.3		
2	24	40.0	20	33.3		
3 - 4	4	6.7	5	8.3		
> = 5	4	6.7	3	5.0	0.62	0.533
Mean ± SD	2 ± 1.6		1.8 ± 1.1			
Median	2.0		1.0			
Serosa	HPAA Count	HPAA Percent	HNAA Count	HNAA Percent	Z#	p
Nil	4	6.7	5	8.3		
1	34	56.7	26	43.3		
2	20	33.3	27	45.0		
3 - 4	2	3.3	0	0.0	1	0.317
> = 5	0	0.0	2	3.3		
Mean ± SD	1.3 ± 0.7		1.5 ± 0.9			
Median	1.0		1.0			

Table 4. Comparison of Muscularis Propria & Serosa With Regard to the Type of Appendicitis by CD 117

Mann - Whitney U Test

Group	Mean ± SD	Median	IQ Range	N	$\chi^2$$	p
Mucosa	3.2 ± 3.6	2.0	2 – 4	60		
Sub mucosa	2.3 ± 1.9	2.0	1 – 2	60		
Muscularis Propria	1.5 ± 1	1.0	1 – 2	60	41.79	p < 0.01
Serosa	1.3 ± 0.7	1.0	1 – 1	60		

Table 5. Comparison of Mast Cell Detection Using Toluidine Blue Staining in Different Layers of Histology-Positive Acute-Appendicitis

Mucosa Vs Submucosa Z# = 2.43*, P = 0.015
Mucosa Vs Muscularis Propria Z# = 4.87, P < 0.01
Mucosa Vs Serosa Z# = 6.09, P < 0.01
Sub mucosa Vs Muscularis Propria Z# = 1.98*, P = 0.048
Sub mucosa Vs Serosa Z# = 3.2**, P = 0.001
Muscularis Propria Vs Serosa Z# = 1.38, P = 0.169
$ Kruskal Wallis Test, # Mann - Whitney U Test
**: - Significant at 0.01 level, * - Significant at 0.05 level

The study compared the mean mast cell distribution by toluidine blue staining and IHC CD 117 in both histology positive acute appendicitis and histology negative cases. Identification of mast cell by IHC staining CD 117 was superior from mucosa and serosa, where as in submucosa and muscularis propria the chance of detecting mast cell was similar.

Group	Mean ± SD	Median	IQ Range	N	$\chi^2$$	P
Mucosa	8.9 ± 4.7	8.0	6 – 12	60		
Sub mucosa	3.5 ± 2.2	3.0	1 - 5.75	60	134.38	P < 0.01
Muscularis propria	2 ± 1.6	2.0	1 – 2	60		
Serosa	1.3 ± 0.7	1.0	1 – 2	60		

Table 6. Comparison of Mast Cell Detection Using CD117 in Different Layers of Histology Positive Appendicitis

Mucosa Vs Submucosa Z# = 7.06, P < 0.01
Mucosa Vs Muscularis propria Z# = 8.8, P < 0.01
Mucosa Vs Serosa Z# = 9.44, P < 0.01
Sub mucosa Vs Muscularis propria Z# = 3.81, P < 0.01
Sub mucosa Vs Serosa Z# = 5.65, P < 0.01
Muscularis propria Vs Serosa Z# = 2.43*, P = 0.015
$ Kruskal Wallis Test, # Mann - Whitney U Test, * - Significant at 0.05 level

Group	Mean ± SD	Median	IQ Range	N	$\chi^2$$	P
Mucosa	3.1 ± 3.6	2.0	2 – 2	60		
Sub mucosa	2.9 ± 2.5	2.0	1 – 4	60	42.79	P < 0.01
Muscularis propria	1.3 ± 1.1	1.0	1 – 2	60		
Serosa	1.2 ± 0.9	1.0	1 – 2	60		

Table 7. Comparison of Mast Cell Detection Using Toluidine Blue Staining in Different Layers of Histology-Negative Acute-Appendicitis

Mucosa Vs Submucosa Z# = 0.01, P = 0.991
Mucosa Vs Muscularis propria Z# = 4.52, P < 0.01
Mucosa Vs Serosa Z# = 4.98, P < 0.01
Sub mucosa Vs Muscularis propria Z# = 4.31, P < 0.01
Sub mucosa Vs Serosa Z# = 4.7, P < 0.01
Muscularis propria Vs Serosa Z# = 0.28, P = 0.779
$ Kruskal Wallis Test, # Mann - Whitney U Test

Group	Mean ± SD	Median	IQ Range	N	$\chi^2$$	P
Mucosa	8.7 ± 5.2	8.5	4 – 12	60		
Sub mucosa	3 ± 2.2	2.0	2 – 4	60	116.7	P < 0.01
MP	1.8 ± 1.1	1.0	1 – 2	60		
Serosa	1.5 ± 0.9	1.0	1 – 2	60		

Table 8. Comparison of Mast Cell Distribution Using CD117 in Different Layers of Histology Negative Appendicitis

Mucosa Vs Submucosa Z# = 6.49, P < 0.01
Mucosa Vs MP Z# = 8.42, P < 0.01
Mucosa Vs Serosa Z# = 8.74, P < 0.01
Sub mucosa Vs MP Z# = 4.13, P < 0.01
Sub mucosa Vs Serosa Z# = 4.99, P < 0.01
MP Vs Serosa Z# = 0.77, P = 0.443
$ Kruskal Wallis Test, # Mann - Whitney U Test

DISCUSSION

In this study, 120 clinically diagnosed cases of appendicitis were divided into histopathology positive and histology negative appendicitis and the mast cells distribution was compared in both groups. Most common age group affected from the data were 11 - 20 year and paediatric age group were affected more. This age distribution pattern is comparable with Usha Rani Singh et al.[5] and Kasper et al.[6] Predominant population were males, which is comparable with Usha Rani Singh et al.[5] Kasper et al[6] and Kumar D et al. also showed similar sex distribution pattern.

Mast cell distribution of mucosa in histologically positive acute appendicitis and in histologically negative appendicitis in toluidine blue staining showed a comparable value (P - 0.49) in both groups. In CD 117 staining, similar finding with P value of 0.030, which is significant in histology negative acute appendicitis. These findings are similar to Usha Rani Singh et al. study. Zhongbo Yang,[7] MD et al. studied mast cell distribution in histologically normal appendicitis and normal control groups of appendices

which was removed incidentally during other surgeries and showed significant increase in mast cells in histology negative acute appendicitis cases in all layers and maximally in mucosa.

Identification of mast cell by IHC staining CD 117 was superior from mucosa and serosa in other similar studies, IHC stain mast cell tryptase was used by Zhongbo Yang,[7] MD et al. and Nigar Coskun N.[8] CD 117 showed strong cytoplasmic and membranous positivity. Toluidine blue staining highlighted mast cell granules. Compared with IHC stain, the quantitative estimation of cells is difficult with toluidine blue, especially in highly cellular areas.

Appendicitis is a common indication for intra-abdominal surgery. The characteristic histologic finding of acute appendicitis is neutrophilic infiltration. A portion of appendices that are resected for a clinical diagnosis of acute appendicitis shows absence of neutrophilic infiltration. The reason for the lack of characteristic histologic findings of acute appendicitis in clinically diagnosed cases is not clear. A few studies have shown that mast cell distribution was higher in histology negative cases than in the normal controls,[9] suggesting that mast cells may play an important role in the pathogenesis of histology negative cases.[10]

In the current study, we were able to show that the mast cell distribution in mucosa, submucosa, muscularis propria and serosa in histologically negative acute appendicitis were significantly higher and comparable to histologically positive acute appendicitis with toluidine blue staining and CD117. By CD 117, the mean distribution of mast cell in mucosa is significantly more in histology negative acute appendicitis compared to histology positive acute appendicitis which suggested a possible role of mast cells in development of symptoms in histology negative cases. On comparing mean mast cell distribution by toluidine blue staining and IHC CD 117, identification of mast cell by IHC staining CD 117 was superior from mucosa and serosa, were as in submucosa and muscularis propria the chance of detecting mast cell was similar.

Although most studies found higher mast cell distribution in histology negative cases than in normal controls, mast cell distribution showed variation in different studies. Some studies showed that mast cell distribution was the highest in the submucosal layer, whereas others showed that mast cell distribution was highest in the mucosa.[11] The current study showed that mast cells have the greatest density in the mucosa in histology negative cases. Hence, it is advisable that mast cells be counted in the appendiceal mucosa in cases of HNAA. HNAA cases with increased mast cells may be reported as appendicitis with increased mast cells, which suggests that the appendectomy is the appropriate treatment and it is not necessary to look for other causes of acute abdomen in such cases.

In human gastrointestinal system mast cells are closely opposed to nerves, with the highest level in the appendix. There is evidence of increased nerve proliferation and hypertrophy in cases of acute appendicitis. The increased mast cell distribution in acute appendicitis may be responsible for nerve proliferation and hypertrophy in acute appendicitis. In an animal study histamine, the major chemical component of mast cells, increased mast cell distribution and induced neuronal hypertrophy in appendix. This indicates that neural hypertrophy and mast cells may play a role in pathogenesis of appendicitis like pain in patients with histology negative cases.[12]

Mast cells are involved in the local regulation of immune events.[13] There is evidence that these cells might be essential for nerve growth and repair.[14] Mast cells, by their numbers, distribution and content of chemical mediators, might influence neural functions and nerve remodeling.[15] Moreover, host response during inflammation needs coordinated neuroimmune interactions between mast cells and nerves.[16] Mast cells contribute to neuroimmune reactions in diseases which results in inflammation of various organs. In the gastrointestinal system, interactions between mast cell and enteric nervous system has been described in various physiologic and pathologic conditions.

CONCLUSIONS

This study concluded that mast cells play an important role in clinical symptoms of patient even when there was no feature of acute inflammation. In those cases, mast cells can be demonstrated by simple toluidine blue staining or IHC markers. There was significant mast cell distribution in all layers of histology negative cases in comparison with cases of histologically positive cases. Thus, mast cells can be considered as an inflammatory indicator in cases where neutrophilic inflammation is absent and appendicectomy is done for acute symptoms. Simple histochemical stain toluidine blue can be used in routine identification and quantification of mast cell. Immunohistochemical markers can be used as a better indicator. The degree of distribution of mast cells have a relation with clinical findings and it is a useful diagnostic marker for histological diagnosis of acute appendicitis.

Limitations

1. The mast cell distribution in normal appendix is not included and compared in this study
2. Long term follow-up of patients with histology negative appendicitis is not done as it is important that there were studies showing relationship between mast cells and inflammatory bowel disease or mastocytosis.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
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