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Research Article

Distribution of Collagen Type I and III and Matrix Metalloproteinase-1 Expression in Cases of Intestinal Tuberculosis

Minakshi Gulia[1]*, Kiran Mishra[1], Neelam Wadhwa[1], Satender Singh[1] and B K Jain[2]

[1]Department of Pathology, University College of Medical Sciences and Guru Teg Bahadur Hospital, New Delhi, India

[2]Department of Surgery, University College of Medical Sciences and Guru Teg Bahadur Hospital, New Delhi, India

***Corresponding Author**: Minakshi Gulia, Department of Pathology, University College of Medical Sciences and Guru Teg Bahadur Hospital, New Delhi, India.

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Abstract

Background: India, being a developing country, has an enormous disease burden of tuberculosis. Average prevalence of all forms of tuberculosis in India is estimated to be 5.05 per 1000 and tuberculosis of GI tract remains the sixth most frequent form of extra-pulmonary TB. Mycobacterium tuberculosis (TB) destroys normal Collagen in lung tissues causing cavitation and this immunopathology is mediated in part by Matrix Metalloproteinase-1 (MMP-1), the Collagenase. In the intestine MMP-1 has also been implicated in the pathogenesis of Inflammatory Bowel Disease, especially Crohn's disease. There are no studies, however, regarding the role of MMPs-1 in intestinal TB. A cross-sectional study was carried out in a tertiary care center to study the distribution of Collagen type I and III and expression of MMP-1 in intestinal tuberculosis.

Materials and Methods: In 30 cases, with clinico-pathological diagnosis of intestinal TB and their resected ends (relatively normal areas), collagen distribution was studied using Picrosirius red staining. MMP-1 expression was determined using Streptavidin biotin method.

Results: Total percent collagen was increased in the submucosa ($p = 0.005$) in lesional areas as compared to resected ends, along with disarray of collagen fibrils. Collagen type I and III was laid down at the periphery of granulomas, leading to encapsulation. Confluent granulomas, however, showed reduced deposition of collagen.

MMP-1 expression was increased in the granulomas ($p < 0.01$), most of them showing moderate to intense staining. Fibroblasts, giant cells, epithelioid cells, macrophages and inflammatory cells had an increased MMP-1 expression in the lesional area. Cases with perforation showed an inverse correlation between collagen distribution and MMP-1 expression, although it was not statistically significant ($p = 0.964$).

Conclusion: Intestinal TB shows an increased MMP-1 expression. There is an increased deposition of Collagen type I and III in the areas of granulomas possibly in an attempt to contain the infection. Confluent granulomas showed less collagen and were seen more in cases of perforation. MMP-1 increases in the areas of granulomas and may play a role in their modulation.

Keywords: *Gastrointestinal Tuberculosis; Matrix Metalloproteinase; Immunohistochemistry; Polymerase Chain Reaction*

Introduction

India is classified to have a high tuberculosis burden and the least prospects of a favorable time trend [1]. Over a fairly long period of observation there is a downward trend and serious escalation of the disease burden.

As per 2009 estimates of global disease burden of Tuberculosis: 9.4 million incident cases (range, 8.9 million–9.9 million) and 14 million prevalent cases (range, 12 million–16 million). According to World Health Organisation (WHO), the incidence of tuberculosis in India

in 2009 was 1.92 million – 2.88 million. Out of these the number of extra-pulmonary cases were 233,026 [7]. The number of prevalent cases in India ranges from 1.56 million – 6 million. Abdominal tuberculosis constitutes upto 12% of extra-pulmonary TB and 1-3% of the total TB cases [2-9].

Being a developing country this disease here is associated with poverty, deprivation, illiteracy, overcrowding and limited access to healthcare facilities.

The disease burden in India is enormous and abdomen remains a major extra-pulmonary site for Tuberculosis. Tuberculosis of the gastrointestinal tract is the sixth most frequent form of extra-pulmonary tuberculosis after, lymphatic, genitourinary, bone and joint, miliary and meningeal tuberculosis [2].

In gastrointestinal tuberculosis Mycobacterium reaches the GIT via haematogenous spread, ingestion of infected sputum or direct spread from infected contiguous lymph nodes and fallopian tubes [3].

It is characterised by transverse ulcers, fibrosis, thickening and strictures of the bowel wall, enlarged and matted mesenteric lymph nodes, omental thickening and peritoneal tubercles [3].

Matrix Metalloproteinases are a family of proteolytic enzymes performing multiple roles in the normal immune response to infection like leukocyte recruitment, cytokine and chemokine processing, activation of defensins and matrix remodeling [4]. However excessive MMP activity following infection leads to immunopathology causing persistence and dissemination of infection [2-4].

In tuberculosis of lung extracellular matrix breakdown with cavitation plays a major role in transmission of bacteria [6]. Experimental studies have evidence of role of MMP-1 secreted by macrophages and expressed by inflammatory cells, in causing local tissue destruction. Tensile strength of the lung is mainly ascribed to type 1 collagen and MMP-1 degrades it at neutral pH [6]. Some recent studies in transgenic mice conclude the role of MMP-1 in causing alveolar destruction and degrading collagen type 3 present around pulmonary tuberculous granulomas, hence aiding the dissemination of the disease. Collagen type 1 is broken down to gelatin which is further degraded by MMP-9 [6].

However, it is noteworthy that *M. Tuberculosis* infection of lung increases MMP-1 and MMP-9 expression and secretion with no compensatory increase in the secretion of Tissue Inhibitor of Matrix Metalloproteinase-1 (TIMP-1) [6].

In relation to GIT there have been studies on pathogenesis of Inflammatory Bowel Diseases. These studies showed an enhanced expression of MMP's, gelatinase A, gelatinase B and collagenases in the regions of fibrosis as well as mucosal degradation in patients of Ulcerative Colitis and Crohn's disease [8].

There are no studies on increased expression of MMP's and possible degradation of various types of collagen by them in human intestinal tuberculosis.

In view of the above stated facts an analytical study was conducted to see whether MMP-1 degrades collagen and drives the immunopathology in human intestinal tuberculosis.

Methods

A cross-sectional study was carried out in the Pathology department of a Tertiary Care General Hospital (University College of Medical Sciences and Guru Teg Bahadur Hospital) in East Delhi, North India.

A review of Histopathological records over 5 years yielded 233 patients with clinical and morphological features of intestinal tuberculosis. Out of these 233, thirty cases were selected in an attempt to balance the sex ratio (1.01), age groups (2nd and 3rd decade), site of involvement (mainly ileum) and to keep an approximately equal number of perforations and strictures. All of the patients selected for this

study underwent resection of the bowel (n = 30; 100%). In some patients continuity of the bowel was restored while in others ileostomy was performed.

Among the intra-operative findings, bowel perforation was the most common (n = 15; 50%). Out of these 15 perforations, 7 were associated with stricture whereas the rest were primary perforations. Strictures were found in 13 (43.3%) patients, including 7 with associated perforations. A total of 12 (40%) patients had ulcers in association with the above findings, whereas 2 (6.7%) patients had only isolated ulcers. Tubercles were found in 11 (36.7%) patients.

Criteria for selection included resected specimens of intestine with transverse ulcers, strictures and with presence of caseating granulomas, resected specimens of intestine with AFB positive solid granulomas and mesenteric lymph nodes/omentum with caseating granulomas.

Fourteen of the 30 cases had definite evidence of tuberculosis in the form of AFB positive cases (3), PCR positive cases (7) and past history of pulmonary TB with AFB positive sputum (Table 1). The remaining 16 cases had caseating granulomas in the intestine and mesenteric lymph nodes.

Evidence	No. of patients (%) n = 30
Caseation/ necrosis	30 (100)
Caseating granulomas in Intestine	28 (93.3)
Caseating granulomas in L. nodes	19 (63.3)
Caseating granulomas only in L. nodes*	2 (6.7)
AFB Positive	3 (10)
PCR positive	7 (23.3)
History of Pulmonary tuberculosis	4 (13.3%)

Table 1. *Evidence of intestinal tuberculosis in 30 patients.*

Collagen was stained by Picrosirius red staining technique and MMP-1 immunostaining was done by Streptavidin biotin method. Polymerase chain reaction (PCR) was used for further evaluation of AFB negative granuloma.

Sirius Red

Sirius red F3B (C.I. 35782): 0.5 g
Saturated aqueous solution of picric acid: 500 ml
Acidified Water: Add 5 ml acetic acid (glacial) to 1 litre of tap/distilled water

Sirius Red Staining Method for Collagen

De-waxed and brought the paraffin sections to water. Stained nuclei with Weigert's haematoxylin for 8 minutes, and then washed the slides for ten minutes in running tap water.

Sections were treated with Sirius red solution for one hour. Washed in two changes of acidified water. Physically removed most of the water from the slides by vigorous shaking. Dehydrated in three changes of 100% ethanol. Cleared in xylene and mounted in a resinous medium. Observed under the microscope in polarized light.

Analysis of Picrosirius Red Staining

In bright-field microscopy collagen is red on a pale yellow background. When examined through crossed polars the larger collagen fibres are bright yellow or orange (Collagen type I), and the thinner ones, including reticular fibres, are green (Collagen type III). The bi-

refringence is highly specific for collagen. A few materials like keratohyaline granules, Type IV collagen in basement membranes and some types of mucus, are stained red but are not birefringent.

For the analysis of Sirius red staining, comparison was done between the lesional area (ulcer/stricture/perforation with granulomas) and the resected ends (relatively normal areas). Resected ends without lesion were available in 22 cases. The remaining 8 cases had granuloma or ulcer and were excluded for comparative analysis with the lesional area.

The percentage of collagen observed in submucosa and serosa as a whole, in cases with stricture as well with perforation, was described. The pattern of collagen distribution and their type, based on the colour of staining, around the tuberculous granulomas was analyzed too.

Streptavidin Biotin Method of Immunostaining

HandE sections of the appropriate block were selected for immunostaining. 3 - 4µ thick sections were taken on lysinated slides, deparaffinized with xylene and rehydrated with graded alcohol and washed with phosphate buffer saline (PBS). Slides were transferred to staining dish containing antigen retrieval solution in antigen retrieving chamber over for 15 - 20 min and washed in PBS. Endogenous peroxide blocking was done by hydrogen peroxide (3%) for 5 min. Slides were washed with PBS for 10 min. Incubation with the primary antibody against MMP-1 for 60 min and washed with PBS for 10 min. Incubation was done with biotinylated secondary antibody for 20 min. Washed in PBS for 10 min. Incubation with preformed avidin-biotinylated peroxidase complex for 20 min and incubation with Diaminobenzidine (DAB) solution for 10 min and rinsed with PBS and then transferred to running water. Counterstaining was done by hematoxylin followed by dehydration, clearing and mounting using DPX.

Polymerase Chain Reaction (PCR): PCR was used for further evaluation of AFB negative granuloma.

Methods for polymerase chain reaction

This set [GeNei™; catalog no. 105935] is based on the principle of single tube nested PCR targeting the IS6110 sequence that is present in multiple copies in the genome of the Mycobacterium tuberculosis complex. Studies have shown that highest sensitivity is obtained by using IS6110 nested PCR when compared to PCR targeting other regions.

Statistical Analysis

The statistical analysis was performed using T-test and Spearman correlation analysis to determine the correlation between expression of MMP-1 and laying down of the two types of Collagen in intestinal tuberculosis. Statistical significance was set at $p < 0.05$.

Results

A total of 233 patients undergoing laparotomy for Intestinal tuberculosis between 2007 to 2012, were analyzed for age and sex distribution, clinical presentation and intra-operative findings.

Age Distribution

The age of patients ranged from 4 years to 75 years. Overall mean age of the patients was 25.87 ± 11.84 years (mean ± S.D). The majority of the patients 71.42% were found to be in their 2nd and 3rd decade of life.

Sex Distribution

The study group consisted of 116 female and 117 male patients. The female to male ratio was 1.01 indicating an almost equal sex preponderance.

Clinical Presentation

In the analysis of a total of 233 patients, the most common presentation encountered was acute perforation peritonitis, documented in 129 patients (55.3%). Intestinal obstruction, seen in 86 patients (36.9%) was the next most common mode of presentation. Eight patients

(3.4%) complained of lump in the abdomen. Pain was a presenting symptom in 99.0% of the patients, followed by constipation (68.5%). Vomiting and abdominal distension was seen in 63.1% and 59.1% of the patients respectively.

Intra-Operative Findings

The most common site found to be involved was small bowel (n = 173; 74.2%). Ileum was involved in 160 (68.7%) patients and jejunal involvement was seen in 26 (12.8%) patients. Thirteen patients had both ileal as well as jejunal involvement. Ileocecal junction was involved in 56 (24%) patients while 1 (0.5) patient had isolated colonic involvement.

Histopathology

The tissues received for histopathology were resected bowel, lymph nodes and omentum. Resected specimens of intestine were available in all 30 selected patients, whereas lymph nodes in 22 (73.3%) and omental biopsy in 9 (30%) cases.

After analyzing the gross findings and Haematoxylin and Eosin stained sections, patients were diagnosed as Granulomatous enteritis (with or without mesenteric lymphadenitis), suggestive of Tuberculosis, based on the presence of:

- Transverse ulcers and strictures with presence of caseating granulomas AND/OR
- AFB positive solid granulomas
- Mesenteric lymph nodes and omentum with caseating granulomas

A total of 28 (93.3%) patients showed presence of caseating granulomas in the resected intestinal segments, whereas caseating lymph node granulomas were seen in 19 (63.3%) cases. Two (6.7%) such cases were reported which had no evidence of granuloma in the resected bowel segment but showed the presence of caseating granulomas suggestive of tuberculosis in the mesenteric lymph nodes.

Results of Sirius Red Staining

Sirius red stained sections were examined under polarized light and the findings were observed based on the color of collagen fibers (Figure 1a to 1d). The percentage of collagen content in the submucosa, in cases with perforation and in cases with stricture, was studied separately.

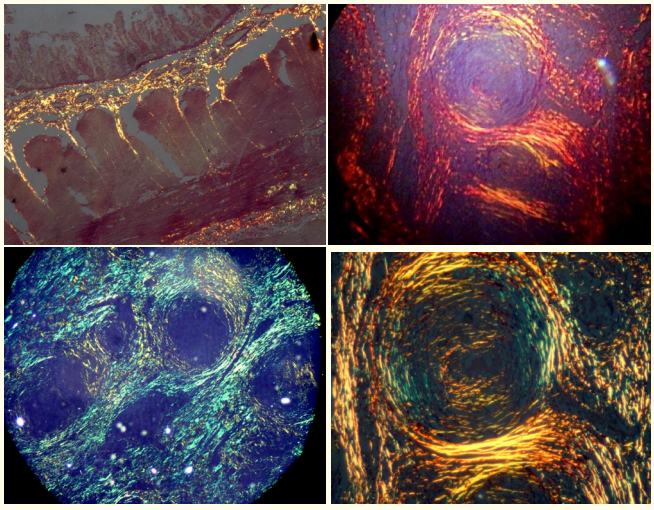

Figure 1a: *PSR staining, resected end (10X): Yellowish orange to yellow (Collagen type I) fibers.*

Figure 1b: *PSR staining, lesional area (20X): Red to reddish orange collagen (Type I) encapsulating the granulomas.*

Figure 1c: *PSR staining, lesional area (4X): Predominantly green (Collagen type III) fibers encapsulating the granulomas.*

Figure 1d: *PSR staining, lesional area (20X): A mixture of yellowish orange and green fibers (Collagen type I and III resp.)*

Out of 30 cases, 6 (5 with perforation and 1 with stricture) showed predominantly green to greenish yellow fibers (Figure 1c) and the rest (80%) had predominantly yellowish orange to red (Figure 1b and 1d) fibers.

Thirteen (43.3%) cases with stricture and their corresponding resected ends (Figure 2a, b, c and d) were examined. Out of 13, only 8 of these resected ends were relatively normal (without granuloma). All 13 cases (lesional areas) showed increased laying down of collagen in the submucosa when compared to the resected ends ($p = 0.005$), specifically around the granulomas, leading to their well circumscription. The resected ends with granulomas also showed features of the lesional area.

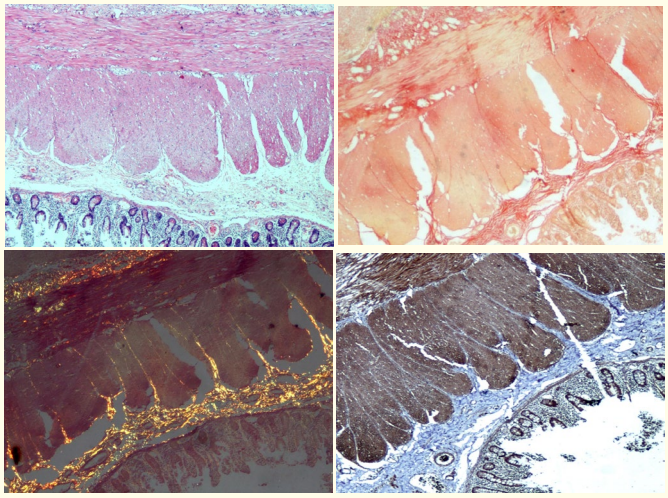

Figure 2a: *Resected end (relatively normal, control). HandE (10X).*

Figure 2b: *Resected end, Picrosirius red (PSR)staining (10X): Collagen fibers staining dark red.*

Figure 2c: *Resected end, PSR staining visualized under polarized light (10X). Submucosa shows well organized distribution of collagen with few fibers dipping into the muscularis propria.*

Figure 2d: *Resected end, immunostaining for MMP-1 (10X). Mucosal epithelium, smooth muscle of blood vessels and muscle layers showing strong expression.*

Most of the granulomas appeared well encapsulated whereas the confluent granulomas showed decreased laying down of collagen. There was marked disarray of collagen fibers in the lesional area, when comparison was done with the resected ends.

Picrosirius red stained sections from 15 patients with perforation and their corresponding (14) resected ends were examined under the polarized light. 100% of the cases showed increased collagenosis of the submucosa ($p = 0.028$) and disarray of collagen fibers. Isolated granulomas had >50% of their circumference encapsulated with collagen fibers (Figure 3a, b, c and d) whereas there was decreased laying down of collagen at the site of granuloma confluence (Figure 4a, b, c and d).

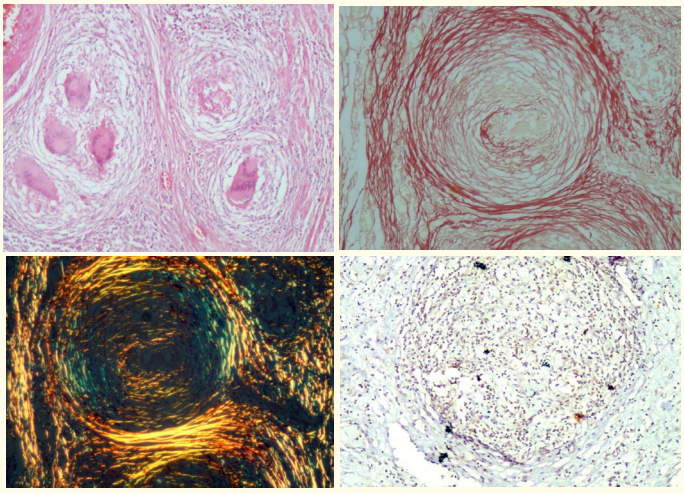

Figure 3a: *Case 1; Section (from AFB positive case), showing mature isolated granulomas with giant cells, HandE (20X).*

Figure 3b: *Case1; PSR staining showing collagen encapsulation (red) at the periphery of granulomas (20X).*

Figure 3c: *Case 1; PSR stained section, visualized under polarized light (20X). A mixture of yellowish orange (Collagen type I) and green fibers (Collagen type III) encapsulating the granulomas.*

Figure 3d: *Case1; Immunostaining for MMP-1. Mild intensity in epithelioid cells of granuloma (20X).*

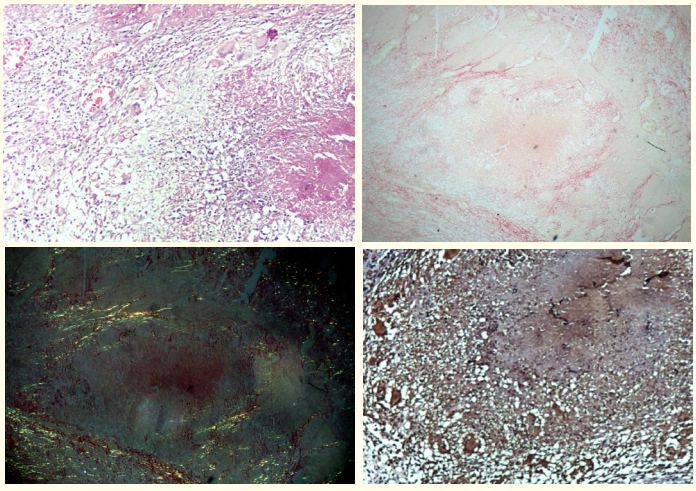

Figure 4a: *Case 2, showing caseating and confluent granuloma in a case of perforation. HandE (20X).*

Figure 4b: *(10X) Case 2, PSR stained section showing red fibers, caseating granuloma and reduced collagen deposition at confluence.*

Figure 4c: *(10X) Case 2, PSR staining, polarization. Markedly reduced collagen fibers (Type III/green/reticulin) around confluent granuloma.*

Figure 4d: *Case2; Immunostaining for MMP-1 (20X). Confluent granuloma exhibiting intense expression in epithelioid cells and Langhans giant cells.*

Results of MMP-1 Immunostaining

All 30 cases, irrespective of their gross features, showed an increased expression of MMP-1 in the region of granulomas (Figure 5a to 5c). 13(43.3%) cases with stricture and 15 (50%) cases with perforation were analysed and compared with their corresponding resected ends (Figure 2d).

In both, cases as well as resected ends, MMP-1 was expressed in the mucosa (epithelial cells), muscularis propria, nerve bundles, fibroblasts, lymphovascular endothelial cells, few lymphocytes and neutrophils. Since tuberculosis is a diffuse process, the resected ends which do not have any lesion, too show increased inflammatory cells and MMP-1 expression by them. There was an increased MMP-1 expression specifically in granulomas, with epithelioid cells, macrophages, giant cells, lymphocytes and fibroblasts showing moderate (Figure 5c) to intense immunostaining.

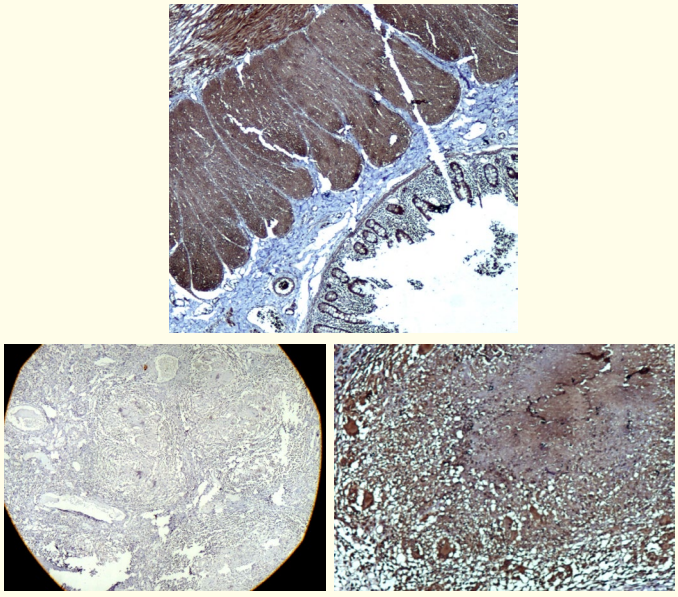

Figure 5a: *Expression of MMP-1, resected end (near normal area, control). Mucosa and muscle layers show strong intensity of staining (10X).*

Figure 5b: *Mild intensity of immunostaining for MMP-1, in lesional area (mature granulomas).*

Figure 5c: *Intense immunostaining for MMP-1 (20X). Langhans giant cells and epithelioid cells seen at the periphery of caseating granuloma.*

Table 2 depicts percentage of MMP-1 expression in submucosa of patients with strictures and perforation versus their corresponding resected ends.

Group	Number	Mean %	S.D	p-value
Stricture	13	55.8	21.3	< 0.01
Stricture, RE	8	23.8	9.2	< 0.01
Perforation	15	50.7	24.4	0.057
Perforation, RE	9	31.1	20.6	0.057

Table 3 depicts percentage of MMP-1 expression in cases (28, 93.3%) versus resected ends (17,56.7%). It signifies an increased expression of MMP-1 in the submucosa of all the cases (53.0+/-22.7%) as compared to their resected ends (27.7+/-16.2%), irrespective of the presence of stricture or perforation. The p-value of 0.000 shows a highly significant correlation here.

Group	Number	Mean %	S.D	p-value
Cases	28	53.0	22.7	< 0.01
Resected Ends	17	27.7	16.2	< 0.01

Correlation of MMP-1 Expression with Distribution of Collagen

Descriptive statistics were used to analyze the correlation between distribution of collagen and expression of MMP-1.

In cases with strictures (13, 43.3%), no significant correlation was derived between collagen and MMP-1 (p-value = 0.337).

Table 4 shows the correlation between MMP-1 and collagen in cases with strictures.

		Stricture, Collagen%	Stricture, MMP1%
Stricture, Collagen%	Pearson Correlation	1	0.29
	p-value	-	0.34
	Number	13	13
Stricture, MMP-1%	Pearson Correlation	0.29	1
	p-value	0.34	-
	Number	13	13

In cases with perforation (15, 50%), an inverse correlation was noted, however it was not significant (p = 0.964).

Table 5 depicts the correlation between percentage of MMP-1 and collagen in cases with perforation.

		Perforation, Collagen %	Perforation, MMP-1%
Perforation, Collagen%	Pearson Correlation	1	-0.013
	p-value		0.964
	Number	15	15
Perforation, MMP-1%	Pearson Correlation	-0.013	1
	p-value	0.964	
	Number	15	15

Discussion

Tuberculosis of the gastrointestinal tract is the sixth most frequent form of extra-pulmonary tuberculosis after, lymphatic, genitourinary, bone and joint, miliary and meningeal tuberculosis.

For the purpose of clinical assessment, a total of 233 cases were analyzed. Majority of the patients in the data reviewed were in their 2nd or 3rd decade of life with a mean age of 29.5+/-12.26 years (Mean+/_ S.D). The maximum incidence of abdominal tuberculosis has been reported between 21 - 40 years and the mean age in Indian population as 26 years [41]. The results of our study suggest an earlier age at presentation than the previous studies. In the present study, the female to male ratio was 0.9:1 which is in agreement with an almost equal incidence to a slight female predominance as stated in the previous studies [42].

Majority of the patients presented in emergency with severe abdominal pain (96.7%), followed by abdominal distension (76.7%) and obstipation (63.3%). Fever (36.7%), vomiting (26.7%), cough (6.7%), weight loss (13.3%) and borborygmi (63.3%) were among the other presenting symptoms reported. This is in accordance with the various studies done on abdominal tuberculosis [1,3,9,10] which also state that abdominal pain is the most common presenting symptom, with incidence ranging from 77 - 94%. In these studies, the incidence of abdominal distension was 28 - 45%, fever 42.2 - 66.2%, weight loss 21.4 - 35%, constipation 24 - 46%, vomiting 33 - 77% and anorexia 10 - 71%. Among the less common but well described symptoms are malabsorption, night sweats, malaise, melena and rectal bleeding.

In most of the studies, the leading acute presentation was intestinal obstruction followed by acute peritonitis [9,10,14,15,18] whereas the most common presentation encountered in our study was acute perforation peritonitis (66.7% patients). Intestinal obstruction (23.3%) was the next most common mode of presentation. This difference in observation could be due to relatively smaller sample size of our study, apart from the difference in time period and socio-geographical aspects of the patient population. The results of our study indicate that majority of patients in our set up present late with complications and do not seek treatment at an early stage of the disease.

In our study, evidence of TB on chest X Ray was reported in 13.3% patients, whereas in previous studies it was seen in 25 - 46% patients [1,3,9,17,43,44]. Free gas under the diaphragm was reported in 33% of the cases in our study, which is in accordance with the previous studies, where it ranged from 38 - 70%.

It has been observed in various studies that ileocaecal region is the most commonly affected site in intestinal tuberculosis owing to various mechanical and pathological factors [1,2,3,7,9,21] whereas few other studies quote small bowel as the most common site [45].

However, our study revealed small bowel to be the most commonly (77.7%) involved site. Ileal involvement was seen in 71.2%, jejunal in 12% and ileocaecal in 21% of patients. Two cases showed isolated caecal involvement (6.7%). Ascending colon, in association with ileum and caecum, was seen to be involving (3.3%) in patients and mesenteric lymphadenopathy in (47.2%) patients. Peritoneal involvement was observed in 30% cases.

The results of the present study indicate significantly higher incidence of small bowel involvement with perforations. This could well indicate that the hospital largely caters to the under privileged population who are malnourished.

In this study, we studied the collagen pattern in cases with stricture and perforation and its correlation with MMP-1 expression. An equal number of cases with perforation and stricture were selected to define the collagen pattern in them and study the simultaneous role of MMP-1 in these areas. There are very few studies in literature explaining the pathogenesis of stricture and perforation in tuberculosis.

Tuberculosis of the intestine generally presents as an emergency with either perforation or ulceration. The healing of ulcer and inflammation lead to stricture, presenting with obstruction. The pathogenesis of ulceration and perforation in tuberculosis have mainly focused on ischemia due to thrombosis of blood vessels. The role of Matrix metalloproteinases and Collagenases and how they act on the collagen in tuberculosis have not been studied.

MMPs are zinc dependent proteases which can effectively degrade all components of the extracellular matrix, including collagen, laminin, fibronectin, vitronectin and proteoglycans. MMP activity is tightly regulated, not only by gene expression, but also by its localization in the cell, its requirement for proenzyne activation and the concurrent expression of tissue inhibitors. MMPs play a role in regulation of immunity, inflammation and host defenses. MMPs through proteolytic cleavage, can modulate the activity of cytokines and chemokines, including IFN-γ, IL-1β, TNF-α, CXCL-8 and CCL-7 [6,24,35].

In various studies on pulmonary TB it has been established that *M. Tuberculosis* drives the expression of MMP-1, which in turn promotes the breakdown of collagen leading to alveolar destruction in pulmonary tuberculosis [6,24,35].

M. tuberculosis infection induces the production of MMPs in vitro in macrophages and in vivo in murine lung tissue.

In a study Elkington., *et al*. [6,35], it was shown that mice treated with BB-94, a broad-spectrum inhibitor of MMPs, exhibited either a delay in granuloma induction or formed smaller granulomas with more collagen. This suggested that MMPs regulate cell migration, lung tissue remodeling and granuloma formation after *M. tuberculosis* infection. MMP–9 from epithelial cell initiates recruitment of new monocytes to the developing granuloma [6,35]. As the granuloma matures Th1 cells, regulatory T cells and B cells are recruited to form a stable granuloma and *M. tuberculosis* persists in a latent stage. A study by Gil., *et al.* [22] concluded that as the granulomas matured, there was a sequential appearance of encapsulation around them (Collagen I and III) in an attempt to contain the infection and establish a latent infection.

During reactivation, granulomas become caseating and necrotic and excessive MMP-1 secretion from the macrophages leads to collagen degradation and tissue destruction (cavitation), which results in *M. tuberculosis* erosion into the airways. MMP-1 degrades both collagen I and III, where collagen I is mainly responsible for the tensile strength of the lung.

In a few studies on Inflammatory bowel diseases too, there was an increased expression of MMP-1 and deposition of collagen in all layers of intestine, especially of collagen type III [30-32], but there have been no studies on expression of MMP-1 in intestinal tuberculosis.

In our study, MMP-1 immunostaining was done on 30 cases of intestinal tuberculosis and their corresponding resected ends (22/30, 73.3%). In both, lesional areas as well as resected ends MMP-1 was expressed in mucosa (epithelial cells), muscularis propria, nerve bundles, fibroblasts, endothelial cells of lymphovascular system, lymphocytes and neutrophils. However, there was an increased expression of MMP-1 in lesional areas with granulomas. Epithelioid cells, giant cells, macrophages, fibroblasts and lymphocytes showed moderate to intense immunostaining for MMP-1. On applying T-test, there was significantly higher expression of MMP-1 in the lesional areas (granuloma, stricture and perforation), 53.04+/-22.7 (Mean+/- S.D), as compared to that in the resected ends, 27.7+/-16.2.

In our study Picrosirius red staining and polarized light were used for qualitative assessment of collagen based on the colour of birefringent fibers and to quantify the collagen content. In Sirius red staining under polarized light, the larger collagen fibers are bright yellow or orange, and thinner ones including reticular fibers are green. Birefringence is highly specific to collagen although fibrin is weakly birefringent [46-51].

Collagen pattern of normal intestinal wall, predominantly submucosa, has been described using Picrosirius red staining [27]. Picrosirius red showed as bright red staining of the collagen fibers in the submucosal layer, which when visualized under polarized light, appeared yellow and yellowish orange [27], in view of the tightly packed and presumable better aligned collagen molecules. Most of the thin fibers in normal submucosa were green and greenish yellow (Collagen type III) (60 ± 9.4%) and the remainder were yellow and yellowish orange (Collagen type I) (40 ± 4.3%) [27].

In the analysis of PSR stained sections from 30 cases (lesional areas) of intestinal tuberculosis, 6 (20%) cases showed predominantly green to greenish yellow fibers (thin reticular fibers/ Collagen type III) and the rest (80%) had predominantly yellowish orange to red coloured collagen fibers (thick fibers/Collagen type I). There was a disarray in the arrangement of collagen fibers and the fibers were haphazardly oriented in the submucosa and serosa. Except 1 case, rest all had > 50% collagenisation of the submucosa and serosa.

Out of a total of 30 resected ends, some showed granulomas and same pattern of collagen deposition as the lesional areas and, hence, were excluded from the comparative analysis. Some of them had a disarray in submucosal collagen distribution despite the absence of granuloma /stricture or perforation. This indicates the diffuse nature of tuberculous pathology.

On applying T-test and comparing the collagen content in submucosa of the selected cases (73.4+/-13.2%) with that of the resected ends (54.3+/-17.4%), there was significantly higher total percent collagen in the lesional areas. Similarly, there was increased total percent collagen in cases with stricture (79.2+/-10.6%) as compared to cases with perforation (68.3+/-13.5%). Both the above findings were proved highly significant statistically (p-value = 0.000).

Conclusion

It may be concluded that intestinal tuberculosis results in increased deposition of collagen in the submucosa and serosa, along with a marked disarray of collagen fibrils. There is more collagen content in cases with stricture as compared to cases with perforation. Collagen is laid down at the periphery of the granulomas, presumably in an attempt to contain the infection or establish a latent infection.

There is a significantly higher expression of MMP-1 in the submucosa and serosa, specifically within the granulomas. Epithelioid cells, fibroblasts, macrophages, giant cells and inflammatory cells show moderate to intense immunostaining for MMP-1, depicting higher

degree of expression. As the maturing granulomas attempt to coalesce, there occurs dissolution of collagen at their periphery, aiding in the confluence. This was evidenced by reduced amount of total collagen in confluent granulomas as compared to mature and solid granulomas.

An inverse correlation was seen between expression of MMP-1 and total percent collagen in cases with tuberculous intestinal perforation signifying that as the level of MMP-1 increases it causes lysis of collagen. A statistically significant value, however, could not be reached as the number of cases was small. Further studies on larger number of cases and quantitative analysis of MMP-1 and collagen are required to confirm the finding that MMP-1 plays a role in lysis of the collagen structure of the intestine. This could be a major breakthrough in the treatment of MDR Tuberculosis as MMP-1 inhibitors (TIMP-1) and the antibiotics with MMP inhibitor activity like Tetracycline and Doxycycline could thus prove beneficial as an adjunct to Anti-tuberculous therapy.

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