INTRODUCTION

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. In humans, it has many clinical manifestations and tends to be chronic. Mycobacteria are acid fast, rod-shaped bacteria having a lipid rich cell wall which retains carbol fuchsin dye even in the presence of acidic alcohol. *Mycobacterium tuberculosis* instead of having a direct toxicity, produces symptoms by mediating the host's immune response. Intestinal tuberculosis is quite common in developing countries on account of poor sanitary conditions, overcrowding and malnutrition. Another contributing factor for its increasing incidence is HIV infection and immunosuppressive treatment. It is predominantly a disease of young adults with almost an equal incidence in both genders.

Intestinal tuberculosis is said to be primary if the gastrointestinal tract is first to be infected by *Mycobacterium tuberculosis*, while it is considered secondary if it occurs as a complication of tuberculosis of some other organ such as lungs. The most common sites of involvement are the colon, the ileocaecal region and the terminal ileum. The gross morphological appearance of tuberculous intestine can be classified into ulcerative, ulcerohyperplastic and hyperplastic. Tuberculous granulomas initially involve the mucosa or the Peyer’s patches of small intestine and tend to be confluent in contrast to those present in Crohn’s disease. Caseating granulomas, usually large, are a diagnostic histopathological finding of intestinal tuberculosis. These consist of a central zone of caseation necrosis, surrounded by epitheloid cells and a peripheral zone which is infiltrated by lymphocytes, plasma cells and Langerhan giant cells. Diarrhoea and constipation are the most common symptoms occurring in intestinal tuberculosis. Intestinal perfusion studies carried out in tuberculosis showed that ileum secreted water and electrolytes while colon showed unimpaired absorption. This indicates that intestinal tuberculosis causes secretory diarrhoea. The ion exchangers, located at the intestinal brush border, play an important role in the pathophysiology of diarrhoea. The latter refers to a term used for an increase in stool mass (upto 250 gms/day in adults is normal), stool liquidity or frequency, osmosis, hypersecretion, exudation and altered motility, all these can cause diarrhoea. A specific diarrhoeal illness can involve more than one of these factors.

It is now known that aquaporins, the water channels, cause diarrhoea by attacking and effacing (collapsing)
bacterial pathogens.\textsuperscript{13} Aquaporin-3, present in colon and small intestine, had been attributed to play a role in constipation/diarrhoea in conditions where vasoactive intestinal polypeptide is secreted as a humoral mediator.\textsuperscript{14} However, aquaporin-3 has a motif YRLL (abbreviation showing the amino acid sequence tyrosine-arginine-dileucine) which is characteristic for basolateral targeting proteins. Aquaporin-10 (AQP-10) lacks this YRLL motif and is, therefore, not located at the basolateral membranes. Aquaporin-10 has three important glycosylation sites. These provide the necessary signals for the location of aquaporin-10 in the apical membranes. This sorting of aquaporin-10 is quite important for intestinal epithelial fluid balance.\textsuperscript{14} The rationale of this study was to investigate the presence/absence of AQP-10 in tuberculous ileum that can be helpful in future in understanding pathophysiology of altered fluid states in tuberculosis. Therefore, the objective of this study was the qualitative analysis of AQP-10 in tuberculous ileum.

\textbf{METHODOLOGY}

It was a cross-sectional analytical study with purposive non-probability sampling done at Department of Anatomy, University of Health Sciences, Lahore, in year 2010. A total of 37 samples were selected from surgically resected specimens/ileal biopsies from cases diagnosed with intestinal tuberculosis and subdivided into two groups. Samples showing granulomatous lesions with or without epithelium (A1) and from the site adjacent to the lesions having intact epithelium (A2). Specimens of ileum with Crohn's disease, malignancy, irritable bowel syndrome, and diarrhoeal diseases caused by Salmonella, Shigella and \textit{Escherichia coli} were excluded.

Routine H&E staining was used to identify and locate the histopathological lesions of tuberculosis in ileum. In addition, Fast Red TR/naphthol AS-MX alkaline phosphatase immunohistochemical technique was used to stain aquaporin-10. Sections of 5 \(\mu m\) thickness were de-waxed in an oven for 20 minutes at 56\(^\circ\)C. Slides were rehydrated and were immersed in phosphate-buffered saline (PBS), after taking them out from 50\% alcohol. Endogenous alkaline phosphatase activity was blocked by covering the sections completely with 1.25 mM solution of levamisole. Non-specific proteins were blocked by treating the sections with 10\% normal goat serum (NGS). The sections were then incubated with primary antibodies (Alpha diagnostic); washed with PBS; treated with secondary antibodies conjugated to alkaline phosphatase; sections were then washed with PBS and treated with the substrate, Fast Red TR/naphthol AS-MX reagent. Lastly, the sections were counter stained with Mayer's haematoxylin and mounted with glycerol gelatin. The slides for negative controls remained untreated with primary antibodies while the remaining procedure was same as for the slides immunostained for aquaporin-10.

The entire slide was scanned through light microscope at different magnifications for the presence/absence and location of AQP-10 which appeared red in positive samples while those without the red colour were considered negative.

Data were analysed by using Statistical Package for Social Sciences (SPSS) version 17. Chi-square or Fisher exact test was applied to find association between qualitative variables. A p-value of \(\leq 0.05\) was considered as statistically significant.

\textbf{RESULTS}

Only 14 (37.84\%) cases of tuberculous group were positive for aquaporin-10 staining. The apical membranes of the cells lining the mucosa were the only sites where aquaporin-10 staining was observed (Figures 1 and 2). A significant association existed between site of granulomas and aquaporin-10 staining (Table I). Out of 37, 23 (62.16\%) tuberculous cases were negative for aquaporin-10 (Figure 3). Out of 23 negative cases, 13 (56.52\%) had transmural granulomas, 6 (26.09\%) had granulomas located in serosa, 3 (13.04\%) showed the granulomas in muscularis externa and serosa. Only one case (4.35\%) was seen with granuloma located in submucosa and serosa. From the total of 37, 14 aquaporin-10 positive tuberculous cases were observed (Figures 1 and 2). Out of 14 positive cases, 11 (78.57\%) showed no granulomas while 1 case (7.14\%) showed granuloma in serosa and 2 cases (14.29\%) had in lamina propria.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{A section from tuberculous human ileum from an area adjacent to lesion showing positive AQP-10 immunostaining (green arrows) at the luminal side of epithelium (E; black arrow) at top of villus. Goblet cells (G; black arrow) with intact apical membrane are positive for AQP-10 (green arrows) while those with ruptured apical membrane are negative for AQP-10 (red arrows). AQP-10 staining is weak in crypts (C; black arrow) and is absent in their deeper parts. X100}
\end{figure}
A significant association was observed between the presence of caseation necrosis and aquaporin-10 staining. Out of 14 aquaporin-10 positive cases, 5 (35.71%) had caseation necrosis (Table I) whereas no caseation necrosis was observed in 9 (64.29%) cases. Of the 23 aquaporin-10 negative cases, caseation necrosis was observed in 19 (82.61%) cases while in 4 cases (17.39%) caseation necrosis was absent.

**DISCUSSION**

The significance of aquaporins in diseases is becoming evident especially in relation to their localization.16 As aquaporin water channels are not active transporters, the driving force in the form of various solutes must be there to move water through these water channels. The most important ions in this regard are Na⁺ and Cl⁻.17,18 Altered gastrointestinal ion transport is seen in both infective and non-infective diseases of the gastrointestinal tract.18 Since water follows the osmotic gradient in various cell lines via paracellular and the aquaporin water channels, it can lead to a number of pathologic conditions affecting gastrointestinal tract characterized by disrupted fluid transport.19

No data appeared in the literature on the expression of water and nutrient channels and transporters in ileal tuberculosis which is characterized by complications as obstruction, perforation, malabsorption and altered bowel habits.5,6,10 The present study was an attempt to localize the aquaporin-10 in tuberculous ileum and to shed light on the controversy of aquaporin-10 localization originally observed in epithelial cells of the luminal part of the villus by Mobasheri et al. and thereafter, by Li et al., who demonstrated the presence of two aquaporin-10 isoforms;20,21 the first one located in gastroentero-pancreatic endocrine cells and the second, AQP-10v, in capillary endothelial cells of duodenum.20,21 Laforenza et al. reported the localization of aquaporin-7 instead of aquaporin-10 on the apical aspect of superficial epithelial cells of the villi of small and large intestines in rats and suggested its involvement in rapid fluid transport across epithelium.22 In another experimental study carried out in rats by Laforenza et al., aquaporin-6 was located on the apical pole of epithelium, and its up-regulation was observed by feeding the rats.23 Later on, Laforenza et al. also reported presence of aquaporin-10 in the brush-border of the absorptive epithelial cells of human duodenum.24 In the present study, out of a total of 37 (100%) tuberculous cases, 23 (62.2%) were negative and 14 (37.8%) were positive for aquaporin-10 staining (Figures 1-3) on the brush-border of epithelium and goblet cells having intact apical membranes. The staining was positive on the mucosa of tuberculous ileum lying adjacent to the site of lesion though it was absent at sites where the underlying granulomatous lesions had disrupted the

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**Table 1: Association between site of granulomas, caseation necrosis and Aquaporin-10 staining.**

<table>
<thead>
<tr>
<th>Site of granulomas</th>
<th>Aquaporin-10</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative n (%)</td>
<td>Positive n (%)</td>
</tr>
<tr>
<td>Transmural</td>
<td>13 (35.12%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Serosal</td>
<td>6 (16.2%)</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>Muscularis and serosal</td>
<td>3 (8.1%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Submucosal and serosal</td>
<td>1 (2.7%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Lamina propria</td>
<td>0 (0.0%)</td>
<td>2 (5.4%)</td>
</tr>
<tr>
<td>Devoid of granuloma</td>
<td>0 (0.0%)</td>
<td>11 (29.3%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>23 (62.15%)</td>
<td>14 (37.85%)</td>
</tr>
</tbody>
</table>

Fisher’s exact test = 14.993, p = 0.002

**Caseation necrosis: Present**

<table>
<thead>
<tr>
<th></th>
<th>Negative n (%)</th>
<th>Positive n (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 (51.4%)</td>
<td>5 (13.5%)</td>
<td>24 (64.9%)</td>
<td></td>
</tr>
<tr>
<td>4 (10.8%)</td>
<td>9 (24.3%)</td>
<td>13 (35.1%)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>23 (62.2%)</td>
<td>14 (37.8%)</td>
<td>37 (100.0%)</td>
</tr>
</tbody>
</table>

Pearson Chi-square = 8.397, p = 0.006
mucosa. The cases with positive staining showed the location of aquaporin-10 on the apical membranes of epithelial cells and goblet cells lining the villi; however, the staining was interrupted at sites where the goblet cells looked clear with plasmalemma ruptured at its apical part, apparently, in the process of pouring out their secretion (Figure 2); also the staining gradually decreased as we proceeded towards the deeper parts of crypts where it was absent. It is believed that in small intestine, most of the secretory activity is performed at the crypts while absorption takes place at the villi.  

It has been observed that aquaporin-10 is normally present on the luminal plasma membrane of the intact epithelium. This is collaborated structurally and functionally with the earlier investigations and seemingly it is established to be helpful in absorption of water. The present investigations substantiate the findings of Sharma and Bhatia and those of Mobasheri et al. and Laforenza et al. However, our findings differ from those reported by Li et al. (2005), who observed the presence of two aquaporin-10 isoforms; one located in gastroentero-pancreatic endocrine cells and the other (AQP-10v) in capillary endothelial cells.  

Most of the cases, which were negative for aquaporin-10 staining, were having a transmural granulomatous lesion (13 out of a total 37; 35.12% cases), the reason being again the involvement of lining mucosa by tuberculous lesion which had spanned through all the layers of the wall of intestine. Its absence may lead to altered bowel habit and malabsorption due to non-absorption of water and small solutes. Most of the aquaporin-10 positive cases (11 of 37, 29.73% cases) showed no granuloma and the overlying mucosa was intact. This part of mucosa may play a role in absorption of water and small solutes through aquaporin-10, or as indicated by Davis et al. may lead to secretory diarrhoea.  

Despite the fact that the present study confirmed the findings of the previous works and provided an evidence for the location of AQP-10 on the mucosa of tuberculous ileum, it still remains to clarify further the role played by aquaporin-10 in the course of tuberculosis. Further, there is a need to investigate how the other aquaporins are affected by tuberculosis, especially aquaporin-3, 9 and 8, in playing their role in the development of diarrhoea. The role played by different aquaporins in pathogenesis of tuberculosis needs to be investigated further, both in primary and secondary type of intestinal disease.

CONCLUSION

Aquaporin-10 staining was found only on the epithelial cells occurring at the luminal side of the villi and was absent in tuberculous ileum where epithelium was absent. Its presence in the sections of tuberculous ileum at site adjacent to the granulomatosus lesion indicated that aquaporin-10 of the adjacent normal looking mucosa is presumably involved in the absorption of water and small solutes or in secretory diarrhoea observed in intestinal tuberculosis.

REFERENCES


