ABDOMINAL ULTRASONOGRAPHIC FINDINGS IN TYPHOID FEVER: A COMPARISON BETWEEN TYPHOID PATIENTS AND THOSE WITH NON-TYPHOIDAL *SALMONELLA* AND *CAMPYLOBACTER JEJUNI* ENTEROCOLITIS

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Abstract. Typhoid fever is a major health problem in many developing countries and its clinical features are similar to other types of bacterial enterocolitis. Definitive diagnosis by blood culture requires several days and is often unfeasible to perform in developing countries. More efficient and rapid diagnostic methods for typhoid are needed. We compared the pathological changes in the bowel and adjacent tissues of patients having typhoid fever with those having bacterial enterocolitis using ultrasonography. A characteristic of patients with non-typhoidal *Salmonella* and *Campylobacter jejuni* enterocolitis was mural thickening of the terminal ileum; only mild mural swelling or no swelling was observed in patients with typhoid fever. Mesenteric lymph nodes in patients with typhoid fever were significantly more enlarged compared to patients with other types of bacterial enterocolitis. Our findings suggest typhoid fever is not fundamentally an enteric disease but rather resembles mesenteric lymphadenopathy and ultrasound is a promising modality for diagnosing typhoid fever in developing countries.

Keywords: typhoid fever, mesenteric lymph node, ultrasonography

INTRODUCTION

Typhoid fever is an acute systemic febrile infection caused by *Salmonella enterica* serovar Typhi (*S*. Typhi). *S*. Typhi is a type of *Salmonella enterica* (*S*. *enterica*) but does not elicit the typical enterocolitis caused by non-typhoidal *Salmonella* strains (House *et al,* 2001; Parry *et al,* 2002). *S*. Typhi evades triggering an innate immune response in the gut of its human host using a stealth approach to allow colonization of deeper tissues in the body (Weinstein *et al,* 1998; Merrell and Falkow 2004). This property may contribute to the unique findings associated with typhoid fever, such as the absence of local inflam-

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mation characterized by the lack of overt thickening of the bowel.

Typhoid fever is commonly associated with systemic manifestations, such as progressive fever, leukopenia, bradycardia, rose spots and splenomegaly, rather than regional intestinal inflammation. Since humans are exclusively susceptible to *S*. Typhi infection, which implies a difficulty in establishing *in vivo* models, its unique pathogenic properties compared to those of other *S. enterica* strains have not been fully elucidated (House *et al*, 2001; Bhan *et al*, 2005). *S. enterica* serovar Typhimurium (*S*. Typhimurium) mainly causes locally restricted enteritis in humans without eliciting a systemic disease. In contrast, oral infection of susceptible mice with *S*. Typhimurium, but not *S*. Typhi, leads to a fatal systemic disease resembling that experienced by humans and is thus used as a model of human typhoid fever. Most research on typhoid fever pathogenesis has been based on *in vitro* studies using *S*. Typhi or *in vivo* studies using *S*. Typhimurium as a substitute bacterial agent (Voedisch *et al*,2009).

Several studies have reported ultrasonographic (US) findings in typhoid fever. Puylaert *et al* (1989) reported US findings in three patients from the United States with typhoid fever, revealing enlarged mesenteric lymph nodes (MLNs) and mural thickening of the terminal ileum. These observations led to the conclusion the findings in typhoid fever are similar to those of non-typhoidal *Salmonella*, *Campylobacter jejuni*, and *Yersinia enterocolitica* cases. Following this study, Tarantino *et al* (1997) evaluated the clinical application of the signs of bowel wall thickening and/or enlarged MLNs to diagnose typhoid fever by assessing the sensitivity (68%) and specificity (81%) of these findings in febrile patients. In these patients, mural thickening (4-9 mm) was observed in only 36.8% of patients with typhoid fever. Nakachi *et al* (2003) reported the clinical findings in the early diagnosis of typhoid fever, emphasizing the usefulness of detecting mesenteric lymphadenopathy with ultrasound as a diagnostic method. Mateen *et al* (2006) demonstrated finding splenomegaly, hepatomegaly and a thick-walled gallbladder are also useful for diagnosing typhoid fever; in all cases of typhoid fever examined they found bowel wall thickening and enlarged MLNs and noticed the five-layered intestinal wall structure was preserved, suggesting minimal destruction.

A recent study (Voedisch *et al*, 2009) reported the use of a mouse model to study *S*. Typhimurium infection; they found MLNs comprised a vital barrier against systemic *S*. Typhimurium dissemination. Since humans are exclusively susceptible to *S*. Typhi, it is important to determine whether morphological changes in MLNs and the degree of enterocolitis seen in mouse models are also seen in patients with typhoid fever. We used US of the abdomen to study culture-confirmed typhoid fever cases, in particular bowel wall thickening and MLN hypertophy, and compared these finding with the US findings of those with other enterocolitic bacterial infections. We evaluated the relationship between US findings and typhoid fever pathogenesis.

MATERIALS AND METHODS

Patients

Fourteen patients were diagnosed with typhoid fever in Peshawar-kai Medical Service Hospital, Peshawar City, Pakistan from July 2000 to June 2001. All cases of *S*. Typhi infection (7 males, 7 females; mean age, 10 years; range, 4-23

years) were confirmed by positive blood cultures. Abdominal US examination was carried out in each patient. The duration of fever prior to US examination had a range of 3-17 days.

Retrospective clinical and US finding among enterocolitis (non-typhoid) patients treated at Kishiwada-Tokushukai Hospital (Osaka, Japan) from 2004 to 2005 were used for comparison. Twenty non-typhoidal *Salmonella* cases (7 males, 13 females; mean age, 26.8 years; range, 1-77 years) and 19 *Campylobacter jejuni* cases (9 males, 10 females; mean age, 22.2 years; range, 2-80 years) were confirmed by stool culture. This study was approved by the research ethics committee of Kishiwada Tokushu-kai Hospital (Osaka, Japan). Informed consent was obtained from all adult participants and from parents or legal guardians of minors. The data were analyzed anonymously.

Modalities

A Capasee PVG-366M diagnostic ultrasound (Toshiba Medical Systems Corporation, Otawara, Japan) with a 5.0 MHz convex and 7.5 MHz linear array transducer was used to examine patients at Peshawa-kai Medical Service Hospital. A Aplio SSA-770-A diagnostic ultrasound (Toshiba Medical Systems Corporation, Otawara, Japan) with a 5.0 MHz convex and 7.5 MHz linear array transducer was used at Kishiwada-Tokushukai Hospital.

All US examinations at both hospitals were conducted by abdominal US experts. After the upper abdomen was examined, the ileocecal region was examined using the graded compression method described by Puyleart (1986). The graded compression method is widely used to evaluate pain in the right lower abdomen. Inflammatory conditions, including thickened bowel loops, and location of thickening, were recorded. Wall thickness of the terminal ileum and proximal colon were measured with firm, momentary compression of the abdomen when no peristalsis was observed.

The five-layered intestinal wall structure observed by US and the method used to measure the mural thickness of the bowel are shown in Fig 1. A similar method was used to evaluate the MLNs. When MLNs were detected, the number and long-axis diameter of the largest MLN were determined in all cases.

Statistical analyses

Correlation coefficients were calculated to determine the relationship between abdominal US findings and fever duration in typhoid fever patients. An unpaired *t*-test was used to evaluate differences in US findings between typhoid and bacterial enterocolitis patients.

RESULTS

In 5 of 14 typhoid cases, mural thickness assessment of the terminal ileum was inadequate. Mural thickness was adequately evaluated in 9 typhoid cases. MLNs were detected by US in all typhoid cases. Enlarged MLNs with oval or round shapes in a cluster or with a beaded appearance were detected as shown in Fig 2.

We evaluated the correlation between duration of fever and mural thickening of the terminal ileum in typhoid cases (Fig 3). The duration of fever prior to US examination ranged from 3 to 17 days. The correlation coefficient (γ) between mural thickening and duration of fever was 0.58, a moderate positive correlation. All cases with mural thickening of the terminal ileum had prolonged fever (>10 days) and mural thickening of the colon. Colon abnormalities were not detected

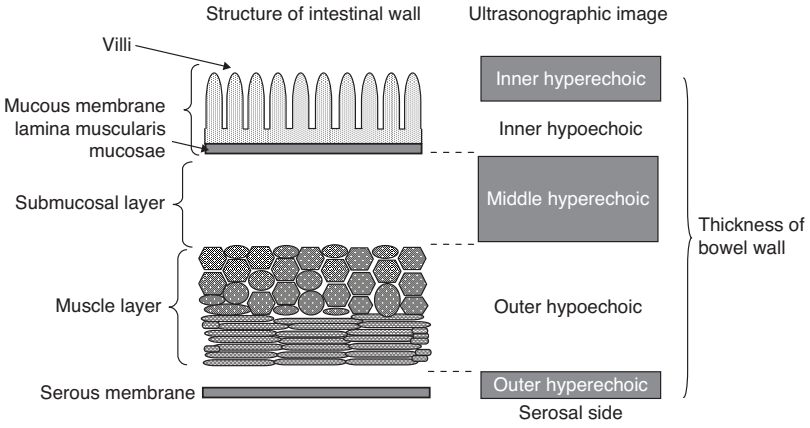

Fig 1–Five-layered structure of bowel wall detected by ultrasonography. The thickness of the bowel wall was measured as the distance between the middle of the inner hyperechoic layer and the outer margin of the outer hyperechoic layer.

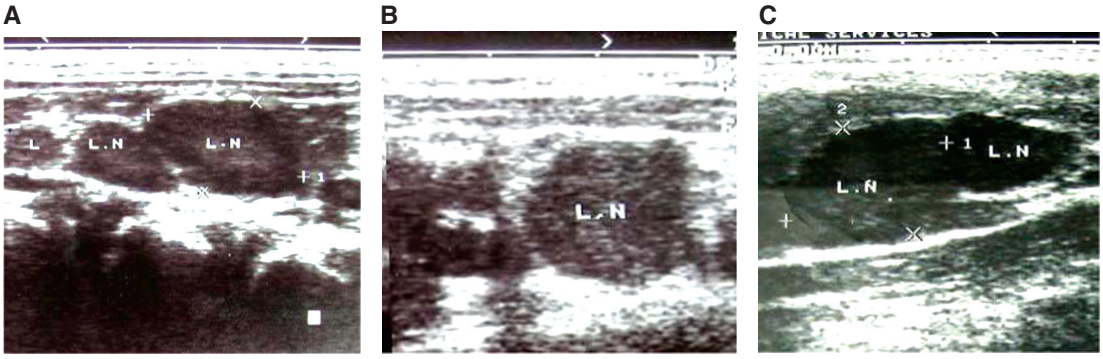

Fig 2–Longitudinal view of the right lower quadrant in three different culture-confirmed typhoid fever cases with enlarged mesenteric lymph nodes. (A) Oval shape, (B) round shape, and (C) beaded appearance.

in cases without mural thickening of the terminal ileum. Next, we assessed the correlation between maximal MLN size and febrile period in typhoid cases; no positive correlation was found. Enlarged MLNs appeared even in typhoid cases shortly after onset; and cases with prolonged fever tended to have MLNs of smaller size (Fig 4).

Mural thickening of the colon was detected in cases with non-typhoidal enterocolitis (6 of 20 non-typhoidal *Salmonella* cases and 7 of 19 *Campylobacter* cases) but mural thickening of the terminal ileum was not detected. Mural thickness of the terminal ileum was evaluated in 26 *Salmonella*/*Campylobacter* cases. Mural thickening of the terminal ileum

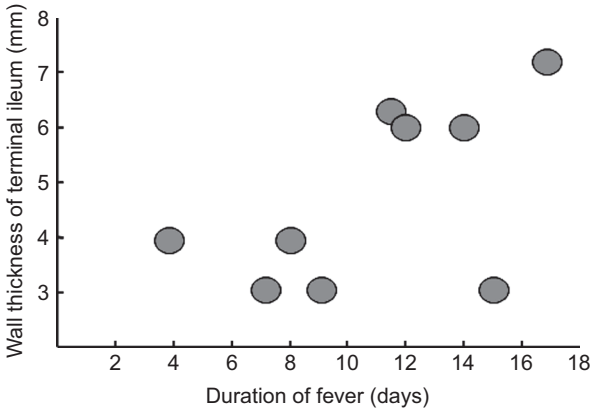

Fig 3–Correlation between mural thickness of the terminal ileum and duration of fever in a typhoid fever case.

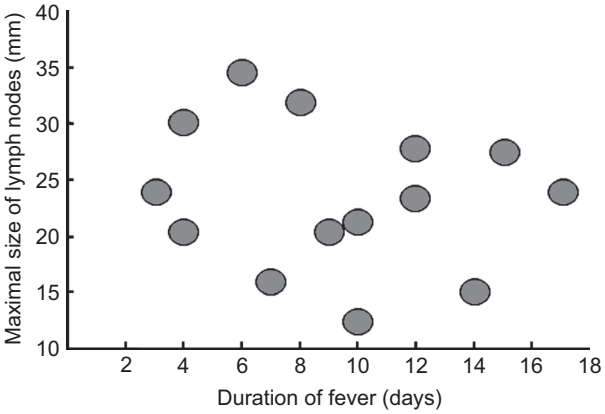

Fig 4–Correlation between maximal lymph node size and duration of fever in a typhoid fever case.

and sigmoid colon and enlarged MLNs in culture-confirmed non-typhoidal *Salmonella* cases are shown in Fig 5. Mural thickening of the ileum and iliocecal valve and enlarged MLNs in culture-confirmed *Campylobacter jejuni* cases are shown in Fig 6. MLNs were not clearly visualized in one non-typhoidal *Salmonella* case and two *Campylobacter* cases. These cases were excluded from statistical analysis.

We compared differences in mural thickening of the terminal ileum between typhoid fever cases and non-typhoidal *Salmonella*/*Campylobacter* cases (Fig 7). Non-typhoidal *Salmonella*/*Campylobacter* cases exhibited significantly greater mural thickening than typhoid cases. Mural thickness of the terminal ileum in typhoid and non-typhoidal *Salmonella* and *Campylobacter* cases were 4.7 ± 1.6, 11.8 ± 2.8, and 9.8 ± 2.3 mm (mean ± standard deviation), respectively. Many of the non-typhoidal *Salmonella* and *Campylobacter* cases exhibited mural thickening of the colon. Compared to non-typhoidal *Salmonella* and *Campylobacter* cases, typhoid cases had significantly larger MLNs (Fig 8). The mean maximal MLN sizes in typhoid, non-typhoidal *Salmonella* and *Campylobacter* cases were 23.3 ± 6.6, 14.3 ± 5.4, and 12.6 ± 4.9 mm, respectively. There was no significant difference in maximal MLN size between non-typhoidal *Salmonella* and *Campylobacter* cases.

The systemic manifestations and prominent MLNs are more characteristic of typhoid fever, than other types of bacterial enterocolitis. Our mural thickening findings suggest typhoid fever should not be classified as a type of bacterial enterocolitis.

DISCUSSION

S. Typhi evades triggering an innate immune response in the gut of its human host using a stealth approach to allow colonization of deeper tissues in the body (Weinstein *et al*, 1998; Merrell and Falkow 2004). Vi capsular polysaccharide (Vi) was first identified as a virulence antigen in *S. typhi*. Vi downregulates early inflammatory responses from intestinal epithelial cells during *S*. Typhi infection (Sharma and Qadri, 2004). These properties may

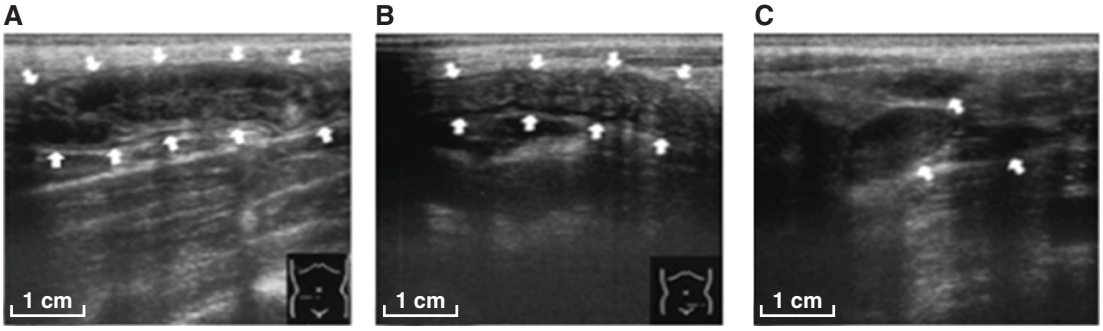

Fig 5–Culture-confirmed non-typhoidal *Salmonella*. (A) Longitudinal view of inflamed terminal ileum, (B) longitudinal view of inflamed sigmoid colon, and (C) enlarged mesenteric lymph nodes.

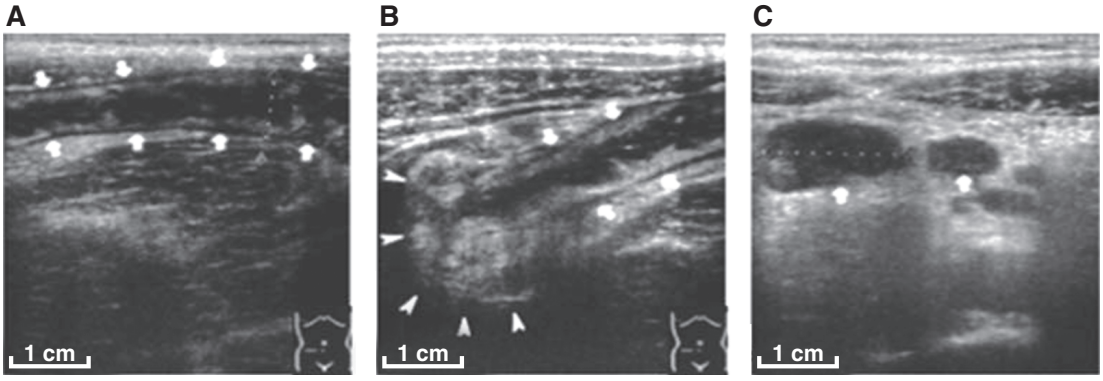

Fig 6–Culture-confirmed *Campylobacter jejuni*. (A) Longitudinal view of inflamed ileum, (B) longitudinal views of an inflamed iliocecal valve (arrow heads) and inflamed ileum (arrows), and (C) enlarged mesenteric lymph nodes.

contribute to the unique findings associated with typhoid fever: the absence of local inflammation characterized by the lack of overt thickening of the bowel. This is in marked contrast to the findings in other forms of salmonellosis involving severe intestinal inflammation. Once ingested, *S*. Typhi enters the small intestine and via M cells of the Peyer's patches, migrates into MLNs where proliferation occurs (Everest *et al*, 2001). Based on this understanding, early diagnosis of typhoid fever in endemic areas by US-based detection of enlarged MLNs may be useful.

Our data demonstrate typhoid fever is more akin to mesenteric lymphadenopathy, rather than an enteric disease. The most important and characteristic US finding in typhoid fever is an enlargement of MLNs with or without mild thickening of the terminal ileum. Mateen *et al* (2006) found the five-layered intestinal wall structure was preserved, which suggests minimal intestinal destruction. Puylaert *et al* (1989) reported sonographic findings in patients with typhoid fever as enlarged MLNs and mural thickening of the terminal ileum. The mural thickening may

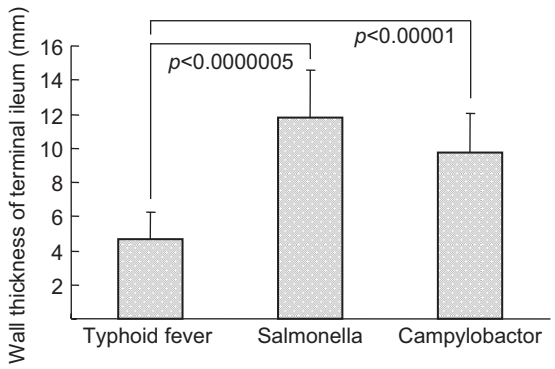

Fig 7–Mural thickness of the terminal ileum in a typhoid fever case.

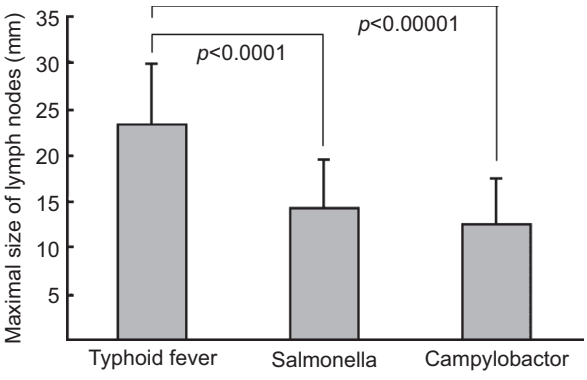

Fig 8–Mesenteric lymph node enlargement in a typhoid fever case.

be an inflammatory response triggered by systemic inflammation, which was observed after the onset of typhoid fever.

We speculate enlarged MLNs may give rise to the systemic manifestations observed with typhoid fever. Voedisch *et al* (2009) postulated a protective role for MLNs in dendritic cell (DC)-mediated dissemination of bacteria in a mouse model using *S*. Typhimurium infection. These regional lymph node-confined DCs loaded with bacteria are considered to be pivotal players in the establishment of adaptive immune responses. Using an *in vitro* human typhoid fever model, Salermo-Goncalves *et al* (2009) demonstrated induction of immunity to *S*. Typhi, or "suicide dendritic cell cross-presentation." In this mechanism, DCs play a pivotal role in priming CD8(+) cells and releasing interferon gamma. These enlarged MLNs may reflect a subsequent immune reaction that involves priming of CD8(+) cells and interferon gamma release by activated DCs.

Although early diagnosis of typhoid fever is crucial for improving prognosis, the nonspecific nature of its clinical features makes diagnosis difficult, since other febrile infections, such as malaria and extrapulmonary tuberculosis, are also found in developing countries (Duggan and Beyer, 1975; Johnson and Aderele, 1981). Definitive diagnosis by blood culture requires at least several days and is often unfeasible to perform in developing countries. With this study, in typhoid fever cases enlarged MLNs appeared shortly after fever onset (Fig 4). In developing countries, US machines are becoming more widely available and mobile machines have become available (Richter *et al*, 2003). The use of US in the early diagnosis of typhoid fever in developing countries may become more common. In this study, we used US to assess typhoid fever pathogenesis and determined typhoid fever is not an enteric disease, but rather resembles mesenteric lymphadenopathy; ultrasound is a promising modality for diagnosing typhoid fever in developing countries.

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