

# Polymerase chain reaction based diagnosis of primary gastric tuberculosis in an 80-year-old woman: a case report and review of the literature

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## SUMMARY

The patient had a two month history of gastrointestinal symptoms. Upper gastrointestinal endoscopy disclosed 5 mm nodular lesions were seen in the prepyloric area. On pathological examination, two granulomatous lesions were detected in biopsy specimen. Ehrlich Ziehl-Neelsen staining and cultures of the biopsy material were negative, but polymerase chain reaction (PCR) for *Mycobacterium tuberculosis* complex DNA was positive. Clinical diagnosis of primary gastric tuberculosis (PGTb) was supported by positive PCR assay and histopathological findings. After antituberculosis treatment, nodular lesions were not detected. The diagnosis of PGTb was confirmed definitively by the success of treatment and repeated endoscopic examination.

**KEY WORDS:** Primary gastric tuberculosis, *Mycobacterium tuberculosis* complex, Polymerase chain reaction

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An increased incidence of extrapulmonary tuberculosis (Tb) has been observed in recent years, especially in populations with immigrants from undeveloped and developing countries and patients with the acquired immunodeficiency syndrome (Baylan *et al.*, 2004; Erdem *et al.*, 2005; Salpeter *et al.*, 1991). Isolated gastrointestinal-Tb (GI-Tb) representing about 3% of all cases of Tb remains a problem today. Only about 50% of these cases are diagnosed accurately, mainly because Tb is often not considered in the diagnosis (Salpeter *et al.*, 1991).

Gastric Tb is quite rare, both as a primary or secondary infection, compared with other sites in the GI tract, and has been reported to occur in

0.5% to 3% of all cases of GI-Tb and is often due to pulmonary infection (Salpeter *et al.*, 1991). According to Palmer (Palmer, 1950) GI-Tb is usually secondary to a pulmonary lesion and he found an incidence of 0.05% involvement of stomach in pulmonary Tb. As with other forms of isolated GI-Tb, its incidence is probably increasing (Salpeter *et al.*, 1991).

The rarity of gastric Tb is due to the presence of gastric acid, continuous motor activity of stomach and the sparsity of lymphoid tissue in the upper GI tract (Salpeter *et al.*, 1991; Amarapurkar *et al.*, 2003; Gupta *et al.*, 1990). Gastric Tb typically centers in the lesser curvature of the antrum and often involves the duodenum (Rathnaraj *et al.*, 1997).

Herein we report a case of an 80-year-old woman with primary gastric tuberculosis (PGTb). The patient was admitted to our clinic with complaints of nausea, vomiting, anorexia, regurgitation, weight loss, fatigue, fever and abdominal pain for the last two months. She did not have cough, shortness of breath, change in bowel

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habits, night sweats or pyrexia. On physical examination there was no palpable lymphadenopathy or hepatosplenomegaly.

Complete blood cell counts and routine serum biochemical parameters were within normal limits. Anti-human immunodeficiency virus (HIV) antibody test was negative. Faecal occult blood test was negative. The erythrocyte sedimentation rate (ESR) (Westergren) was 22 mm/hour and C-reactive protein (CRP) was 18 mg/L at admission. Results of other laboratory tests, i.e., coagulation tests, urine analysis, tumor markers, levels of vitamins as folate and B<sub>12</sub> and thyroid hormones were normal.

The immune status of the patient was investigated and results of tests were found normal limits. The diagnosis could not be established with the history, physical examination and biochemical analyses of the patient.

It was noted that the corpus and antrum of the stomach were edematous and hyperemic, 5 mm nodular lesions had swollen the mucosal layer of prepyloric area at the upper GI endoscopy (Figure 1). Biopsies were taken from the nodular lesions and stomach mucosa that appeared normal in the upper GI endoscopy. It was detected that there were two granulomatous lesions in the pathological examination of biopsy specimen. The pathologists advised evaluating the patient for granulomatous diseases and Tb.

Gastric biopsy specimens taken from lesions were also evaluated mycobacteriologically for PGTb. Ehrlich Ziehl-Neelsen (EZN) staining of the biopsy material was negative for acid-fast bacilli (AFB), and culture results of the biopsy material were negative for conventional (Löwenstein Jensen, Salubris Inc., Istanbul, Turkey) and radiometric (BACTEC 460 TB culture system, Becton Dickinson Diagnostic Instruments, Sparks, MD, USA) methods.

A standard protocol for extraction and amplification of *Mycobacterium tuberculosis* complex (MTC) DNA from the gastric biopsy specimens was performed. Briefly, biopsy specimens were minced and pretreated by gentle mixing with 50 µl of proteinase K (100 µg/ml) and 200 µl of buffer (0.01 M of Tris-HCl [pH:7.8] (10 mmol Tris-HCl), 0.005 M of EDTA (20 mmol EDTA), 0.5% SDS), and subsequent incubation at 40°C overnight, then boiled for 10 minutes. Phenol-chloroform extraction was performed, and the

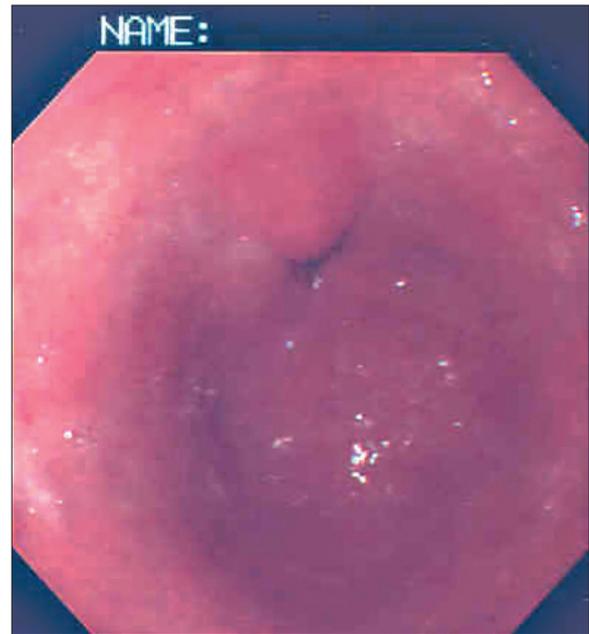


FIGURE 1 - Nodular hyperemic lesion (3 mm) in the prepyloric area of antrum.

ethanol-precipitated DNA was resuspended in TE buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 7.4) and used for amplification. The primer sets used to amplify the 123-bp IS6110 gene fragment consisted of TBC1 (5'-CCT GCG AGC GTA GGC GTC GG-3') and TBC2 (5'-CTC GTC CAG CGC CGC TTC GG-3') (Baylan *et al.*, 2004; Erdem *et al.*, 2005). The amplification reactions were performed using Taq polymerase and reagents according to the manufacturer's instructions (Bioron GmbH, Biotechnica Co., Ludwigshafen, Germany). Five microliters of DNA were added to the mixture, which was subjected to 40 cycles of amplification (95°C, 30 sec.; 68°C, 30 sec.; 72°C, 30 sec.) followed by a 5 minutes extension at 72°C (Thermocycler; MJ Research, Watertown, Mass., USA). Fifteen microliters of the amplification products were analyzed by electrophoresis carried out in ethidium bromide-stained 2% agarose gel, and the results were visualized by ultraviolet light. Finally, MTC DNA was detected in the gastric biopsy specimens by polymerase chain reaction (PCR). X-ray images were obtained and computed tomography (CT) scans of the pulmonary and genitourinary systems were performed, and these imaging studies disclosed no evidence of primary

foci of the gastric Tb. There was no history of pulmonary, GI or genitourinary Tb. There was no known family history of Tb. She denied any knowledge of previous tuberculin skin tests. Various specimens including sputum and urine were examined with EZN staining, cultures and PCR for the presence of mycobacteria; all of these test results were negative. Purified protein derivative (PPD) testing was not reactive, with a 4 mm induration at 72 hours.

The suspected clinical diagnosis was supported by positive PCR assay and histopathological findings despite negative AFB and culture results. Anti-Tb treatment was started orally with four drugs, isoniazid (INH) (300 mg/day), rifampicin (RMP) (600 mg/day), ethambutol (EMB) (1.5 g/day) and pyrazinamide (PZA) (2 g/day) during the first two months, followed by INH and RMP for four months. High dose pyridoxine (10 mg/day) was prescribed to prevent INH-related neuropathy during treatment.

The patient tolerated the anti-Tb treatment well and no complications occurred. Treatment resulted in complete resolution of the signs and symptoms of disease. At the follow-up oesophagogastroscope after anti-Tb treatment, nodular lesions were not detected and the patient in this case had a dramatic response to anti-Tb treatment alone. ESR and CRP returned to normal ranges. Three months after the anti-Tb therapy, she was still symptom-free. Finally, the diagnosis of PGTb was confirmed definitively by the treatment success and repeat endoscopic examination. This is the first such case report.

In the first half of the last century, GI-Tb could occur in the absence of additional sites of infection because of the ingestion of infected milk with *Mycobacterium bovis*. Bovine GI-Tb has now been essentially eradicated in countries with milk pasteurization and tuberculous testing of dairy herds (Salpeter *et al.*, 1991). Although the ileum, colon and the ileocecal region are commonly infected sites, any part of the GI system may be infected (<http://www.emedicine.com/radio/topic885.htm>). These areas can be reached with a flexible endoscope (<http://www.emedicine.com/radio/topic885.htm>; Amarapurkar *et al.*, 2003). An awareness of these atypical presentations of Tb is necessary for the proper diagnosis and prompt institution of therapy (Salpeter *et al.*, 1991; Segal *et al.*, 1981).

There has been much debate regarding the origin of GI infection with *M.tuberculosis*. Three theories may play a role in causing GI-Tb. These are direct ingestion, hematogenous spread, and lymphatic spread. Lymphatic spread would occur by transluminal transport of the tubercle bacillus to the submucosal lymph nodes with subsequent colonization and the formation of granulomas. This theory is supported by the fact that most cases of GI-Tb occur in areas rich in lymphatics, such as the ileocecal region. It has been hypothesized that the infection remains dormant in the mesenteric lymph nodes with reactivation and spread at a later date (Salpeter *et al.*, 1991).

Isolated and PGTb without evidence of lesions elsewhere is uncommon (Amarapurkar *et al.*, 2003). Okoro and Komolafe (Okoro&Komolafe, 1999) reported two patients with gastric Tb with unusual presentations. One of the patients was an elderly man suspected to have abdominal malignancy but subsequently found to be extensive, complicated gastric Tb coexisting with chronic peptic ulcer disease. The second patient was a woman who developed gastro-bronchial fistula due to Tb which was evident radiologically (Okoro&Komolafe, 1999). A report by Chetri K *et al.* (Chetri *et al.*, 2000) has shown a case of gastric Tb presenting as non-healing gastric ulcer.

Common symptoms include fatigue, weight loss, fever, and abdominal pain (Salpeter *et al.*, 1991). The clinical manifestations of GI-Tb are non-specific; a high index of suspicion is therefore important to ensure a timely diagnosis; missed or delayed diagnosis could lead to increase of morbidity and mortality (Horvath&Whelan, 1998). Symptoms of stomach involvement include abdominal pain and upper GI bleeding. Nausea and vomiting is a feature when gastritis and outlet obstruction are present (<http://www.emedicine.com/radio/topic885.htm>). Post prandial pain in the abdomen, distension and discomfort, vomiting and loss of weight may be the presenting features of these patients (Wani *et al.*, 1977). Amarapurkar *et al.* published five gastric Tb patients with these symptoms (Amarapurkar *et al.*, 2003). Presenting symptoms are often related to gastric outlet obstruction or similar to those of peptic ulcer disease and rarely with haematemesis (Rathnaraj *et al.*, 1997).

Gastric Tb may show multiple large and deep ulcers in the stomach, most frequently on the less-

er curvature of the antrum or in the pyloric region. Scarring from ulcers leads to diffuse antral narrowing resulting in gastric outlet obstruction. The stomach may be diffusely involved and show irregular contour, simulating a linitis plastica of primary scirrhous carcinoma of the stomach. Multiple fistulous tracks may develop as the disease advances (<http://www.emedicine.com/radio/topic885.htm>).

Lesions may be ulcerative, hypertrophic or a combination of the two, called ulcerohypertrophic. On pathological examination of the endoscopic biopsy, granulomas are generally present in the submucosa and subserosa, with a nonspecific inflammatory reaction in the mucosa (Salpeter *et al.*, 1991). The complications of these lesions are haemorrhage, perforation, obstruction and fistula formation (Wani *et al.*, 1977). Ulcerative lesions are the commonest; the others are hypertrophic lesions, miliary tubercles and tuberculoma. Ulcers, single or multiple, and hypertrophic nodular lesions surrounding a stenotic pyloric channel are described. On biopsy, granulomas are either caseous or non-caseous (Rathnaraj *et al.*, 1997).

Gastric Tb is usually associated with pulmonary Tb or with immunodeficiency state (Amarapurkar *et al.*, 2003). Approximately 20-25% of patients with GI-Tb have pulmonary Tb (<http://www.emedicine.com/radio/topic885.htm>). Wig *et al.* reported a case of isolated gastric Tb presenting as massive haematemesis. This patient was found to have benign gastric ulcer along the lesser curvature (Wig *et al.*, 2000).

Laboratory findings are nonspecific. Anemia and an elevated ESR could be present in some cases. Tuberculin skin test is positive in about 65% of cases. Radiographic studies may show an ulcer or a submucosal mass (Salpeter *et al.*, 1991).

A definitive diagnosis of gastric Tb usually requires the histological or bacteriological identification of *M.tuberculosis* (Salpeter *et al.*, 1991). Most patients end up with surgical intervention and the diagnosis of gastric Tb is made after surgery because an accurate clinical diagnosis is lacking. The diagnosis of Tb requires demonstration of caseating epithelioid granuloma or presence of acid-fast bacilli in tissue (Amarapurkar *et al.*, 2003). Upper GI endoscopy and biopsy play an important role in diagnosis (Rathnaraj *et al.*, 1997).

PCR is used increasingly in the diagnosis of Tb. PCR of mucosal biopsy specimens diagnoses colonic Tb in 45 to 64% of cases. By use of mucosal biopsies, *Mycobacterium tuberculosis* is demonstrated by culture to occur in one third and by PCR in two thirds of patients with colonic Tb. In countries with a high incidence of Tb, GI-Tb is often diagnosed and treated on empirical grounds and confirmed by resolution following therapy. PCR using *M. tuberculosis* H37Rv, but not atypical mycobacteria, produced a 123-bp amplicon. In a study by Balamurugan *et al.*, the sensitivity of fecal PCR for the diagnosis of intestinal Tb was 88.8%, and the specificity was 100% (Balamurugan *et al.*, 2006). Because biopsy is of limited diagnostic value for intestinal Tb and the differentiation between intestinal Tb from Crohn's disease, PCR may be used to diagnose the intestinal Tb. (Amarapurkar *et al.*, 2004; Gan *et al.*, 2002). This is the first case report because diagnosis of gastric Tb from gastric biopsy material has not been reported in the literature.

Clinically GI-Tb resembles peptic ulcer disease or malignancy in the endoscopy (Amarapurkar *et al.*, 2003). The clinical picture of gastric Tb simulates chronic gastritis, peptic ulcer, inflammatory bowel disease, malignancy, and infectious diarrhea but at times clinical presentation may be misleading (Amarapurkar *et al.*, 2003; Wani *et al.*, 1977). When granulomas are non-caseating, small and discrete, the differential diagnosis on histology includes Crohn's disease, sarcoidosis, syphilis, mycotic lesions and exposure to beryllium, silicates or reserpine (Amarapurkar *et al.*, 2003). Early articles suggested that 10% of cases of gastric Tb were associated with gastric carcinoma, but this correlation has not been verified in recent data (Salpeter *et al.*, 1991). Gastric analysis to differentiate Tb from carcinoma of the stomach has also been of little help (Wani *et al.*, 1977). Parmer (Palmer, 1950) reported achlorhydria in 25% and hypochlorhydria in 57% of patients.

The treatment of gastric Tb is similar to that of pulmonary Tb. Short-course treatment (six or nine months) appears to be effective, with a rapid resolution usually seen in symptoms, fever, and the size of the gastric mass or ulcer. Early suggestions that surgical extirpation of the gastric lesions is essential for cure are no longer substantiated, unless acute complications are present

(Salpeter *et al.*, 1991). Partial gastrectomy followed by anti-Tb chemotherapy has been advised as the treatment of choice (Stirk, 1968).

Though gastric Tb is rare, patients with nodular lesions at upper GIS endoscopy presenting with gastric outlet obstruction or with endoscopic evidence of diffuse chronic inflammatory activity, the possibility of gastric Tb should be kept in mind especially in areas endemic for Tb. PCR of gastric samples has not been validated for the diagnosis of PGTb, but it is very difficult to diagnose PGTb by conventional methods. In our case, it was shown that PCR is a reliable and rapid method for supporting the diagnosis of PGTb, and that it should be used in the routine diagnostic algorithm when conventional methods fail to identify MTC and for the patients at high risk of PGTb. As this is the first case to be positive in gastric mucosal PCR, further studies should be done to determine the efficacy of PCR in the diagnosis of PGTb from gastric mucosal specimens.

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