Endoscopic jejunal biopsy culture: a simple and effective method to study jejunal microflora

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Abstract

Background Jejunal fluid culture is the gold standard for assessing jejunal microflora. Aspiration of jejunal fluid is sometime difficult. As the microorganisms rests on the mucosal surface, culture of the mucosal biopsy may be a possible alternative method.

Aim To study the role of jejunal mucosal biopsy culture to assess jejunal microflora and to compare it with jejunal fluid culture.

Methods Thirty adult subjects with gastroesophageal reflux disease requiring endoscopy underwent enteroscopy. Jejunal fluid aspirate and mucosal biopsy were cultured. The procedure was repeated after omeprazole therapy in 18 patients.

Results Forty-eight pairs (30 preomeprazole therapy and 18 postomeprazole therapy) of fluid and mucosal biopsies were cultured. In 45 of the 48 pairs (94%), both the culture of jejunal biopsy and jejunal fluid yielded similar results with respect to the presence (n=27) or absence of growth (n=18). In the remaining 3 pairs, the growth was present either in the biopsy culture (n=2) or in the fluid culture (n=1) only. Among those pairs in which growth was present, microorganisms isolated were identical in 53%, differed by ≤2 organism in 37% and different by >2 organisms in 10%. Ten of the 12 patients who were detected to have small intestinal bacterial overgrowth (SIBO) on fluid culture were also detected to have SIBO on biopsy culture. Sensitivity, specificity, positive and negative predictive value of biopsy culture in diagnosing SIBO was 83.5%, 97.2%, 94.7%, and 91.6%, respectively.

Conclusion Culture of unwashed endoscopic jejunal mucosal biopsy is an effective and simple alternative to jejunal fluid culture for assessing jejunal microflora.

Keywords Bacterial overgrowth · Enteroscopy · Jejunal microflora · Mucosal biopsy culture

Introduction

Jejunal fluid aspirate culture is the gold standard for studying jejunal microflora and establishing the diagnosis of small intestinal bacterial overgrowth (SIBO) [1]. Jejunal fluid can be aspirated by endoscopic suction, intestinal intubation with a catheter, a capsule biopsy or by intraoperative bowel aspirate [2]. Aspiration with a sheathed sterile catheter during endoscopy is most frequently used to obtain jejunal fluid. However, aspiration of jejunal fluid is sometimes difficult, increases endoscopy time and is at times unsuccessful due to sparseness of jejunal fluid [3]. As microorganisms are predominantly present in the mucus layer which overlies the intestinal epithelium, culture of the mucosal biopsy could be an alternative option to study the jejunal microflora [2].
The results of the few studies which have tried to compare the role of culture of jejunal fluid versus that of mucosal biopsy have been conflicting [2, 4, 5]. These studies had used mucosal biopsies which were washed in order to remove the adherent mucus before culture. However, as organisms may be adherent to the mucus layer, culture of the washed biopsies may be unrepresentative. In this study, we examined the role of culture of unwashed jejunal mucosal biopsy to assess normal jejunal microflora and compared it with jejunal fluid culture.

Methods

Patients with symptomatic gastroesophageal reflux disease (GERD), attending the gastroenterology services of a tertiary care hospital were evaluated. Those requiring upper gastrointestinal endoscopy were included in the study. Patients who were on antibiotics, prokinetics or acid suppressants in the preceding 4 weeks were excluded. Patients with predisposing conditions for SIBO, like diabetes, scleroderma, hypothyroidism, and prior gastric surgery were excluded [6]. Written informed consent was obtained from all patients and the study was approved by ethics committee of our institute.

In all the study subjects, a push enteroscopy with SIF-10, Olympus enteroscope (length 165 cm) was performed. Jejunal fluid aspirate and jejunal mucosal biopsies were obtained in the same sitting. The same procedure was repeated after 6 weeks of omeprazole (20 mg BD) therapy in those who consented for a repeat procedure.

Endoscopic technique

Enteroscope and its attachment were sterilized in 2% glutaraldehyde for 30 min and rinsed with sterile normal saline. All patients underwent enteroscopy after an overnight fast, and fresh sterile gloves were used for each procedure. Minimal air insufflation was used. Jejunal fluid was aspirated from proximal jejunum (50–70 cm from the pylorus) using ethylene oxide sterilized 5 F metal-tipped ERCP cannula. One milliliter (mL) of aspirate was collected in a sterile syringe. The aspirate was immediately transferred into 9 mL Robertson aspirating broth (Marine chemicals, Chullical, India) to give 1:10 dilution. Subsequently, two mucosal biopsies were obtained from the same site with a sterilized multibite biopsy forceps (Microvasive, (Microvasive/Boston Scientific corporation, Massachusetts, USA)) and immediately placed in another tube containing RCM broth, both of which were transported and processed within 30 min. Each biopsy was removed from the RCM broth weighed on a sterile pre-weighted butter paper. The biopsy was homogenized with 1 mL sterile saline and vortexed to obtain a uniform suspension which was used for inoculation. Gastric aspirate was also collected during withdrawal of the enteroscope for pH estimation using micro-pHmeter (Accu pH, Spectralab Instrument Pvt Ltd, Thane, India).

Bacteriological analysis

The aspirated jejunal fluid which was transported in RCM broth (already diluted 1:10) was serially diluted further (1:100, 1:1,000) in phosphate buffer containing 1% peptone. Homogenized biopsy sample was also serially diluted (1:10, 1:100, 1:1,000). 20 μL of a specific dilution each of the fluid/homogenized mixture was inoculated onto 5 separate culture plates. The total bacterial count was assessed using Miles and Misra method [7]. The dilution which yielded countable colonies was used for computing the bacterial counts. The average number of colony counts per plate (i.e., per 20 μL) was calculated for every dilution.

The culture media used for aerobic culture were chocolate agar, 5% sheep blood agar and MacConkey’s medium. The organisms were identified by standard bacteriological techniques [8]. Qualitative anaerobic cultures were done directly from the RCM tube (without dilution) onto 5% sheep blood agar containing haemin (5 μg/ml) and vitamin K (menadione) (1 μg/mL). The plates were incubated anaerobically in an anaerobic jar at 37°C for 48 h.

The total colony forming units (CFU) in jejunal fluid per mL and jejunal biopsy per gram were calculated by the following formulae for jejunal fluid and biopsy, respectively.

\[
\text{No. of CFU per 20 } \mu\text{L of fluid inoculated} = \frac{1}{20} \times \text{dilution} \times 1000
\]

\[
\text{No. of CFU per 20 } \mu\text{L of suspension inoculated} = \frac{1}{20} \times \text{dilution} \times 1000 \times \frac{\text{Weight of the biopsy in gm}}{20}
\]

Cultures were considered positive for SIBO if the total bacterial count was ≥10⁷ CFU per mL of fluid or per gram of biopsy tissue [9].

Statistical methods

All data were analyzed using SPSS version 10 (manufacturer, city of manufacture). Sensitivity, specificity, positive predictive value, and negative predictive value were calculated using appropriate formulae. Normally distributed values were expressed as mean (SD), nonparametric values were expressed as median (interquartile range). Correlation statistics was performed to study the correlation between bacterial counts of total aerobes recovered from fluid and biopsy culture. The differences between baseline and post-omeprazole therapy values of bacterial count and pH were...
determined by using Wilcoxon sign ranks test and paired t test, respectively. An interrater reliability analysis using the Kappa statistics was performed to determine consistency between fluid and biopsy culture for detecting SIBO, presence or absence of any organism, aerobes and anaerobes. Sample size was calculated using web based statistical software. With $\alpha$ error of 5% and $\beta$ error of 10% with an assumed pilot correlation estimate of 0.7, minimum required sample size was 14.

**Results**

Endoscopy was performed and both jejunal fluid and mucosal biopsies were obtained in 30 patients (20 men) with GERD. Mean (SD) age of the patients was 31.3 (6.4) years. In 18 of them, the same procedure was repeated following 6 weeks of omeprazole therapy. Thus, 48 pairs (30 baseline and 18 postomeprazole therapy) of fluid and mucosal biopsies were analyzed. In 45 of the 48 pairs (94%) both the culture of jejunal biopsy and jejunal fluid yielded similar results with respect to presence ($n=27$) or absence of growth ($n=18$). In the remaining 3 pairs, growth was present either in the biopsy culture ($n=2$) or in the fluid culture ($n=1$) only. Among those pairs in which growth was present, microorganisms isolated were identical in 14 pairs (53%), differed by $\leq 2$ organisms in 10 (37%) and different by $>2$ organisms in 3 (10%).

**Comparison of viable microorganisms**

The median (IQR) count in fluid ($n=30$) and biopsy ($n=30$) culture in the patients at baseline was $0$ ($0–1080$) CFU/mL and $0$ ($0–3200$) CFU/gm, respectively. The viable aerobic bacterial counts obtained from fluid and biopsy culture showed a high degree of positive correlation (Spearman’s $\rho=0.862, p<0.001$) (Fig. 1).

The median (IQR) gastric pH increased from 3 (0.93, 4.0) to 4.65 (2.77, 6.45) after six weeks of omeprazole therapy ($p=0.002$). Paired cultures after omeprazole, total counts increased significantly from median (IQR) $0$ ($0, 1080 \times 10^5$) CFU/mL to $1.2 \times 10^5$ (5950, $15.5 \times 10^5$) CFU/mL in fluid culture ($p<0.001$) and from $0$ ($0, 2900 \times 10^5$) CFU/gm to $2.37 \times 10^5$ (6500–$12.5 \times 10^5$) CFU/gm in biopsy culture ($p<0.001$). SIBO was present in one of these patients at baseline. It was detectable on fluid culture only. After omeprazole, SIBO was found in 11 (61%) patients on fluid culture ($p=0.001$) as well as on biopsy culture ($p=0.001$).

The microorganisms recovered from jejunal fluid and biopsy culture are listed in Table 1. The culture of biopsy and fluid yielded similar results with regards to the distribution of both intestinal and oral flora. In all the 5 patients in whom anaerobes were isolated in the jejunal fluid, they were also detected on jejunal biopsy culture. Biopsy and fluid showed agreement with respect to presence or absence of any organism ($k=0.829, p<0.001$). The microorganisms recovered from jejunal fluid and biopsy culture are listed in Table 1. The culture of biopsy and fluid yielded similar results with regards to the distribution of both intestinal and oral flora. In all the 5 patients in whom anaerobes were isolated in the jejunal fluid, they were also detected on jejunal biopsy culture. Biopsy and fluid showed agreement with respect to presence or absence of any organism ($k=0.829, p<0.001$).

### Table 1: Microbial species isolated on fluid and biopsy culture

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Fluid culture</th>
<th>Biopsy culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral flora</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non pathogenic Neisseriae</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Alpha-hemolytic Streptococci</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Coagulase negative Staphylococci</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Candida</td>
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<td>2</td>
</tr>
<tr>
<td>Diphteroids</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Hafophillus</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Flavobacteria</td>
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<td>1</td>
</tr>
<tr>
<td>Intestinal flora</td>
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<td></td>
</tr>
<tr>
<td>Enterococci</td>
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<td>8</td>
</tr>
<tr>
<td>Acinobacter</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
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<td>5</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
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<td>3</td>
</tr>
<tr>
<td>Lactobacilli</td>
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<td>1</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
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<td>1</td>
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<tr>
<td>Citrobacter</td>
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<td>2</td>
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<tr>
<td>Anaerobes</td>
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</tr>
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<td>Peptostreptococci</td>
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<td>Fusobacteria</td>
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<tr>
<td><em>Bacteroides fragilis</em></td>
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<td>2</td>
</tr>
</tbody>
</table>

![Fig. 1](scatterplot.png) Scatter plot shows a high degree of positive correlation between quantitative results obtained on jejunal fluid and biopsy culture ($n=48$). Log transformation of values have been done to log base 10. Spearman’s $\rho=0.862, p<0.001$, CFU: colony forming units. Sixteen pairs of fluid and biopsy culture had no growth in either fluid or biopsy culture and are not represented in the figure.
The median viable counts of *Escherichia coli* recovered from the jejunal fluid showed a high degree of positive correlation with the counts obtained from the jejunal biopsy (Spearman’s rho = 0.849; p = 0.001). The mean number of species isolated from fluid and biopsy per patient were 3.35 (0–5) and 3.31 (0–6), respectively.

Twelve jejunal fluid aspirates had microbial counts (median [IQR] 8.5×10^5 [1.25, 31.8×10^5] CFU/mL) which were suggestive of SIBO. Culture of biopsy detected SIBO in 10 of these 12 patients. One patient had SIBO on biopsy culture but the jejunal fluid culture was not suggestive of bacterial overgrowth. The kappa interrater reliability for jejunal fluid and biopsy culture was 0.79 (p < 0.001). Sensitivity, specificity, positive predictive value, and negative predictive value of culture of biopsy in diagnosing SIBO were 83.5%, 97.2%, 94.7%, and 91.6%, respectively.

**Discussion**

The present study has demonstrated that the culture of jejunal biopsy yields results similar to fluid with respect to presence of growth, nature of organisms, and presence or absence of SIBO. The bacterial counts obtained by both the methods also showed a high degree of correlation. Moreover, culture of biopsy tissue is able to detect the change in nature and amount of flora after omeprazole. In the diagnosis of SIBO, the two techniques showed high degree of interrater reliability.

There are several advantages of using jejunal biopsy for culture, namely sterile biopsy forceps, which are available in all endoscopic suites; mucosal biopsies are routinely obtained in most patients undergoing endoscopy, while performing biopsies is easier, faster, and more efficient than jejunal fluid aspiration. Moreover, the inner cup of the biopsy forceps remains unopened during introduction and thus, theoretically has lesser chances of contamination. Unwashed biopsy was used for culture in the present study. In the only study published in English literature, where culture of unwashed biopsy was compared to luminal fluid, a significant correlation was obtained with respect to the total bacterial counts and type of organisms recovered. Also, culture of biopsy was found to have a high sensitivity (90.3%), specificity (100%), and predictive value (100%) for diagnosing SIBO [3]. These results similar to those obtained in our study.

Previous studies investigating the bacterial flora of mucosal biopsy had used washed mucosal biopsies to remove adherent mucus. Conflicting results regarding the similarity between luminal and adherent small intestinal bacterial ecologies were obtained. These studies also reported preferential isolation of anaerobic organisms on culture of biopsy [2, 4, 5]. Thus, washed mucosal biopsy culture is possibly not representative of microbial flora compared to unwashed biopsies.

Significant increase in bacterial colony count following omeprazole therapy was reported in both fluid and biopsy culture as gastric acid is the major defence mechanism against gut bacteria proliferation. Several other studies have also noted increase in gut bacterial count following omeprazole therapy [10–13]. Patients with predisposing conditions for SIBO were excluded, because in addition to comparing the two techniques, we wanted to study the effect of omeprazole on bacterial flora and these conditions might have played a confounding role.

We have used 1,00,000 CFU/mL as criteria for SIBO as it is the standard criterion for definition [9]. After 6 weeks of omeprazole, we found a significant increase in jejunal bacterial count and 61% were found to have bacterial overgrowth. As expected, the gastric fluid pH was high after omeprazole; the reduction in gastric acid possibly explains the higher number of gut bacteria. Achlorhydria is known to be a predisposing factor for SIBO [14].

In conclusion, culture of unwashed jejunal mucosal biopsy yields organisms representative of those obtained in jejunal fluid culture, with respect to the counts as well as nature of organism. Culture of unwashed mucosal biopsy also had high sensitivity, specificity, and predictive value for detecting SIBO. Thus, culture of mucosal biopsy is a useful and an easier alternative to fluid aspirate culture for studying jejunal microflora and for diagnosis of SIBO.

**References**


