Bacterial Flora Detected of the Uterine Endometrial Cavity of Diabetic Patients with Myoma Uteri

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Abstract

Patients with diabetes frequently suffer from various postoperative complications, especially infection. Diabetic patients also have a high incidence of uterine endometrial cancer. The nature of the intrauterine bacterial flora may be related to both infection and carcinogenesis. Therefore, identification of the intrauterine bacterial flora in diabetic patients may be useful.

Bacteria were detected in the uterine endometrial cavity of 100% of ten diabetic patients with myoma uteri. However, among 20 non-diabetic control patients with myoma uteri, only three 15% harbored bacteria.

Members of the Enterobacteriaceae (Escherichia coli, Proteus spp., Enterobacter cloacae, and Klebsiella pneumoniae) were the predominant bacteria.

We speculate that bacterial products contribute to carcinogenesis, as has been proposed for colon carcinoma. Antimicrobial agents active against Enterobacteriaceae should be used to prevent postoperative infections in gynecologic procedures in diabetic patients.

Introduction

Patients with diabetes frequently suffer from various postoperative complications, especially infections1. Components of the uterine endometrial bacterial flora are considered to be the likely causative organisms of infections after total hysterectomy2.

In addition, the high incidence of uterine endometrial cancer in diabetic patients led us to try to determine the etiological factor in the endometrial cavity. Some bacteria produce carcinogens such as N-nitro compounds3-5, n-butyric acid6,7, and n-valeric acid7, which are products of Escherichia coli3,4, anaerobic bacteria5-7, and others. Uterine cervical cancer is associated with high colony counts of E. coli, Gardnerella vaginalis, Streptococcus agalactiae, Bacterioides sp. and Prevotella sp.8-13. These bacterial species are known to produce carcinogens and stimulators of cell growth15,16.

Therefore, knowledge of the bacterial flora of the uterine endometrial cavity of diabetic patients might provide insight into both carcinogenesis and the development of post-operative infections.
Materials and Methods

Subjects

Ten diabetic patients between 35 and 45 years old (average, 40 years), and 20 non-diabetic patients were studied. Those patients underwent hysterectomy because of myoma uteri in the Department of Obstetrics and Gynecology of the School of Medicine, Gifu University, from June 1989 to July 1992.

Collection and processing of specimens

At total abdominal hysterectomy, samples were immediately taken from the endometrial cavity with a polyester fiber swab (Falcon Applicator, Becton Dickinson, Cockeysville, U.S.A.) and suspended in 5 ml of an anaerobic buffer containing a reducing agent in a CO2-filled tube.

The composition of the anaerobic buffer was as follows: KH2PO4, 4.0 g; Na2HPO4, 6.0 g; L-cysteine • HCl • H2O, 1.0 g; Tween 80 (Sigma Chemical Company, St. Louis, U.S.A.), 1.0 g; and agar, 1.0 g; distilled water, 1,000 ml/pH 7.2. All the components were mixed and a solution was made by heating at 80°C for 30 minutes. Nine-milliliter quantities of the buffer were transferred to test tubes. Immediately after the air in the tubes was replaced with CO2, the tubes were sealed with a butyl rubber stopper. All tubes and solutions were sterilized in an autoclave at 115°C for 20 minutes.

After the samples were suspended in the buffer, the tubes were re-sealed under a continuous stream of carbon dioxide gas of commercial grade to drive the air out. Exposure of the samples to atmospheric oxygen was restricted to 5 minutes or less. Incubation was commenced immediately after the samples were suspended in the solution. A small quantity was aspirated with syringe via a butyl rubber stopper and retained for subsequent quantitative culture.

Quantitative bacteriologic assay

Dilutions of 10^-2, 10^-4 and 10^-6 were prepared with an anaerobic buffer. A 0.1-ml sample from each dilution was plated on each of 8 media. For anaerobic organisms, Brucella HK blood agar (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan), PEA Brucella HK blood agar (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan), PV Brucella HK laked blood agar (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan), and Bacteroides bile esculin agar (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan) were used. These anaerobic agar plates were stored under anaerobic conditions. The following media were used to isolate aerobic and facultative anaerobic isolates: blood agar (Becton Dickinson, Cockeysville, U.S.A.), chocolate agar (Becton Dickinson, Cockeysville, U.S.A.), MacConkey agar (Nissui Pharmaceutical Industrial Co., Tokyo, Japan) and Staphylococcus selective agar (Nissui Pharmaceutical Industrial Co., Tokyo, Japan). Anaerobic cultures were incubated in an anaerobic chamber at 35°C in 5% CO2 for chocolate and blood agar plates and in air for MacConkey agar and Staphylococcus selective agar.

After incubation, CFU (colony forming unit) of different types of colonies on plates from both atmospheres were determined and the bacteria were subcultured on blood agar plates and purified by standard microbiologic methods. Quantitative results were expressed as the range and the mean log/log of viable bacteria per swab.

Identification of isolates

All bacterial isolates were identified at the levels of genus and species. Anaerobic isolates were identified by using the Rap ID ANA II identification system (Innovative Diagnostic System, Inc., Atlanta, GA) combined with gas-liquid chromatography (GLC) for identification of fatty acids that were produced during bacterial growth in PYG broth (Scott Laboratories, Rockville, MD). Isolates that were difficult to identify were tested by techniques described in the Anaerobic Laboratory Manual[17].

Microaerophilic and aerobic isolates were identified by standard identification schemata. Gardnerella vaginalis was identified by using the API STREP identification system (API System S.A., Montalieu
Lactobacillus was identified at the genus level by GLC; members of the family Enterobacteriaceae were identified by using the Enterotube II identification system (Becton Dickinson, Cockeysville, U.S.A.); members of the family Micrococcaceae were identified by using the API STAPH identification system (API System S.A., Montalieu vercieu, France); members of the genera Streptococcus and Enterococcus were identified by the API STREP identification system.

**Results**

Seventeen samples (85%) from the uterine endometrial cavity of non-diabetic patients with myoma uteri were sterile. In the other three cases, Staphylococcus epidermidis at a colony count of $10^{3.5}$, Lactobacillus acidophilus at $10^{3.7}$, and Enterococcus faecalis at $10^{4.1}$ were isolated.

Bacteria were isolated from the uterine endometrial cavity of all ten diabetic patients with myoma uteri (Table 1). Patients 1 and 2 had insulin-dependent diabetes mellitus (IDDM), and patients 3 to 10 had non-insulin-dependent diabetes mellitus (NIDDM). Gram-positive cocci isolated were Staphylococcus haemolyticus, Streptococcus agalactiae and Enterococcus faecalis. Gram-negative bacilli isolated included E. coli in six cases, Citrobacter freundii in one case, Proteus spp. in two cases, Morganella morganii in one case, Enterobacter cloaceae in two cases, and Klebsiella pneumoniae in two cases. The anaerobic bacteria isolated included Bacteroides fragilis, Peptostreptococcus anaerobius, and Peptostreptococcus micros.

There was no difference in the nature of the isolates between IDDM and NIDDM among the patients examined.

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Bacterium isolated</th>
<th>Colony count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Escherichia coli, Bacteroides fragilis</td>
<td>$10^{4.8}$</td>
</tr>
<tr>
<td>2</td>
<td>Streptococcus agalactiae, Lactobacillus acidophilus, Morganella morganii</td>
<td>$10^{4.1}$</td>
</tr>
<tr>
<td>3</td>
<td>E. coli, Enterobacter cloaceae</td>
<td>$10^{4.8}$</td>
</tr>
<tr>
<td>4</td>
<td>E. coli, Klebsiella pneumoniae, Enterococcus faecalis</td>
<td>$10^{4.9}$</td>
</tr>
<tr>
<td>5</td>
<td>Gardnerella vaginalis, K. pneumoniae</td>
<td>$10^{4.0}$</td>
</tr>
<tr>
<td>6</td>
<td>E. coli, Peptostreptococcus anaerobius</td>
<td>$10^{4.9}$</td>
</tr>
<tr>
<td>7</td>
<td>E. coli, Proteus mirabilis</td>
<td>$10^{4.8}$</td>
</tr>
<tr>
<td>8</td>
<td>L. acidophilus, Citrobacter freundii, Peptostreptococcus micros</td>
<td>$10^{4.0}$</td>
</tr>
<tr>
<td>9</td>
<td>E. coli, E. cloaceae</td>
<td>$10^{4.9}$</td>
</tr>
<tr>
<td>10</td>
<td>Staphylococcus haemolyticus, Proteus vulgaris</td>
<td>$10^{4.8}$</td>
</tr>
</tbody>
</table>
Discussion

The uterine endometrial cavity is normally sterile\(^4\). However, the present study shows that in the uterine endometrial cavity of diabetic patients, bacteria are consistently detected.

Gram-negative bacilli, especially *Enterobacteriaceae*, were the predominant bacteria isolated. *Escherichia coli*, *Proteus* spp., *Enterobacter cloacae*, and *Klebsiella pneumoniae* predominated in the uterine endometrial cavity of diabetic patients. Bacteria were easily isolated from the cavity in the immunologically-impaired patients. Diabetes mellitus induces a similar impaired state\(^18,19\).

Epidemiologic risk factors of uterine endometrial cancer include diabetes and obesity\(^20\). Bacteria in the uterine endometrial cavity are likely to produce carcinogens such as N-nitro compounds\(^3,5\), n-butyric acid\(^6,7\), and n-valeric acid\(^7\), or growth factors such as transforming growth factor, insulin-like growth factor, epidermal growth factor, and membrane-mediated growth factor\(^16\).

Since *Enterobacteriaceae* were the predominant microbes in the endometrial cavity of patients harboring bacteria, gynecologic prophylaxis should possibly include agents active against these potential pathogens.

References

糖尿病合併者の子宮内細菌叢についての検討

1) 三鴨 廣繁 2) 和泉 孝治 伊藤 邦彦
渡辺 邦友 上野 一恵 玉宮 輝彦
（平成5年1月21日受付）
（平成5年4月8日受理）

産科婦人科領域の細菌感染症では、上行感染が大部分を占めるため子宮内の細菌叢を知ることは、合併症を起こす主因の一つと考えられる。特に、子宮全摘術後の感染予防における子宮内細菌叢を知ることの重要性は、Empiric therapyを施行するうえで重要なデータとなる。この予防的治療において、糖尿病合併患者の子宮内細菌叢を知ることは合併症のない患者20例、糖尿病合併症で有症患者10例の子宮内細菌叢について検討した。その結果、糖尿病合併症患者の子宮内-Esherichia coli, Proteus mirabilis, Enterobacter cloacae, Klebsiella pneumoniae-などのEnterobacteriaceaeが優位を占めていた。以上より、不幸にして糖尿病合併症を合併した場合には、子宮内細菌叢を発症した場合や、術後骨盤内感染症を発症した場合には、これらの腸内細菌にも強い抗菌作用を示す薬剤を使用する必要があると考えられた。