Endometrial Bacterial Flora Detected in Patients with Uterine Endometrial Cancer

Hiroshige MIKAMO, Koji IZUMI, Kunihiko ITO, Kunitomo WATANABE*, Kazue UENO* and Teruhiko TAMAYA

Department of Obstetrics and Gynecology, School of Medicine, Gifu University
*Institute of Anaerobic Bacteriology, School of Medicine, Gifu University
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Abstract

Certain bacteria produce some carcinogens such as N-nitro compounds, $n$-butyric acid and $n$-valeric acid.

From this point of view, the examination of intrauterine bacterial flora in patients with uterine endometrial cancer may provide important information.

Twenty patients with the diagnosis of uterine endometrial cancer and 20 patients without complications other than myoma uteri were enrolled in the study. Enterobacteriaceae, Streptococcus agalactiae and anaerobic bacteria were mainly detected.

The products of these bacteria might be considered to contribute to the initiation of endometrial carcinogenesis.

Mixed abnormal flora between aerobic and anaerobic bacteria were detected in all patients with uterine endometrial cancer. It is suggested that uterine endometrial cancer provides favorable conditions for bacterial growth. Mixed abnormal bacterial flora also might influence the onset and growth of uterine endometrial cancer.

Introduction

There are three categories of risk factors for uterine endometrial cancer1): the first is what may be considered variations in normal anatomy or physiology; the second is certain frank disease states; the third is exposure to known external causes of disease.

Certain bacteria can produce carcinogens such as N-nitro compounds2)–7), $n$-butyric acid and $n$-valeric acid8)–13). The carcinogens are produced by Escherichia coli2)–6) and anaerobic bacteria7)–13). In uterine cervical cancers, high colony counts of bacteria are detected, including E. coli, Gardnerella vaginalis, Streptococcus agalactiae, Bacteroides sp. and Prevotella sp.14)–19). These bacteria might produce carcinogens or stimulators of cell growth21)–23).

Therefore, it seemed worthwhile to investigate the endometrial bacterial flora in patients with uterine endometrial cancer.
Materials and Methods

Subjects
Twenty patients with the diagnosis of uterine endometrial cancer, aged between 44 and 69 (average: 55.7), and 20 patients without complications other than myoma uteri, aged between 38 and 53 (average: 46.4) were enrolled in the study. The patients with uterine endometrial cancer included stages O, IA, IB, IC, IIA, IIB, IIIA, IVB. They underwent hysterectomy at 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 dissolved into 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; agar, 1.0 g and 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 milliliters of the anaerobic solution was placed into each tube. 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 samples were suspended in the buffer, all tubes were re-sealed under a continuous stream of carbon dioxide gas of commercial grade to drive the air out. The duration of exposure of the samples to atmospheric oxygen was restricted to 5 minutes or less. Cultures were commenced immediately after the bacteria were suspended in the solution.

An aliquot was aspirated by a syringe via a butyl rubber stopper and retained for subsequent quantitative culture.

Quantitative bacteriologic assay
Serial 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 Bacterioides bile esculin agar (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan) were used.

These anaerobic agar plates were stored under anaerobic conditions. The following media were employed for 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 for 7 days. Aerobic cultures were incubated for two days at 35°C in 5% CO₂ for chocolate and blood agar plates or air for MacConkey agar and Staphylococcus selective agar.

After incubation, colony forming unit (CFU) 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 are expressed as the range and mean log₁₀ 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 system II (Innovative Diagnostic System, Inc., Atlanta, GA) combined with gas-liquid chromatography (GLC) to identify the fatty acid that is produced during bacterial growth in PYG broth (Scott Laboratory, Rockville, MD). Isolates that were difficult to identify were subjected to techniques described in the anaerobic laboratory manual. Microaerophilic and aerobic isolates were identified by standard identification schemata. Gardnerella vaginalis was identified with API STREP identification system (API System S.A., Montalieu vercieu, France); Lactobacillus was identified to the genus level by GLC; members of the family Enterobacteriaceae were identified by using the Enterotube II identification system (API System S.A., Montalieu vercieu, France); members of the family Micrococcaceae were identified with the API STAPH identification system (API System S.A., Montalieu vercieu, France); members of the genus Streptococcus and Enterococcus were identified with the API STREP identification system.

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<th>Case #</th>
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<th>Colony count</th>
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<td>Lactobacillus acidophilus</td>
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<td>Enterococcus faecalis</td>
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Table 2

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<td>Bacteroides thetaiotaomicron</td>
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<td>Peptostreptococcus micros</td>
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Table 1 Bacterial flora in the uterine endometrial cavity in patients without complications other than myoma uteri

Table 2 Bacterial flora in the uterine endometrial cavity in patients with uterine endometrial cancer

6. IA  Staphylococcus haemolyticus | $10^{9.8}$ |
7. IB  Peptostreptococcus micros | $10^{9.0}$ |
8. IB  Escherichia coli | $10^{9.8}$ |
9. IB  Enterococcus faecalis | $10^{8.4}$ |
10. IB  Prevotella bivia | $10^{9.0}$ |
11. IC  Streptococcus agalactiae | $10^{9.2}$ |
12. IC  Klebsiella pneumoniae | $10^{9.0}$ |
13. IIA  Prevotella bivia | $10^{9.4}$ |
14. IIA  Escherichia coli | $10^{9.2}$ |
15. IIB  Peptostreptococcus anaerobius | $10^{8.4}$ |
16. IIB  Enterococcus faecium | $10^{9.4}$ |
17. IIA  Enterococcus faecalis | $10^{9.6}$ |
18. IIC  Streptococcus agalactiae | $10^{9.0}$ |
19. IVA  Citrobacter freundii | $10^{9.4}$ |
20. IVB  Bacteroides distasonis | $10^{9.4}$ |
Endometrial Bacterial Flora in Patients with Uterine Endometrial Cancer

Results

Seventeen samples (85%) from the uterine endometrial cavity in patients without complications other than myoma uteri were sterile. In the other three cases, *Staphylococcus epidermidis* was detected at 3.6, *Lactobacillus acidophilus* at 3.5, and *Enterococcus faecalis* at 4.0 were detected (Table 1).

Bacteria were detected in the uterine endometrial cavity in all twenty patients with uterine endometrial cancer (Table 2). Of the gram-positive cocci, *Staphylococcus haemolyticus* was detected in three cases, *Streptococcus agalactiae* in six cases, *Enterococcus faecalis* in three cases and *Enterococcus faecium* in two cases. Of gram-positive bacilli, *Staphylococcus aureus* was detected in one case. Of gram-negative bacilli, *Escherichia coli* was detected in eight cases, *Citrobacter freundii* in two cases, *Proteus* sp. in one case, *Enterobacter cloacae* in two cases, and *Klebsiella pneumoniae* in five cases. As anaerobic bacteria, *Bacteroides fragilis* was detected in four cases, *Bacteroides distasonis* in five cases, *Bacteroides thetaiotaomicron* in two cases, *Prevotella bivia* in seven cases, *Peptostreptococcus anaerobius* in three cases, *Peptostreptococcus magnus* in three cases, *Peptostreptococcus micros* in two cases, and *Propionibacterium acnes* in one case.

Discussion

The uterine endometrial cavity is normally sterile\(^2\). However, bacteria were more frequently detected in the uterine endometrial cavity of patients with uterine endometrial cancer than in those without complications other than myoma uteri. Compared with the bacterial flora in patients with uterine cervical cancer, the endometrial bacterial flora was less in this study.

The types of bacteria predominantly detected were, gram-negative bacilli, especially *Enterobacteriaceae*, and anaerobic bacteria. *Enterobacteriaceae*, such as *Escherichia coli* and *Enterobacter* spp., predominated in the uterine endometrial cavity of patients with endometrial cancer. Bacteria such as *Enterobacteriaceae* and anaerobic bacteria are easily detected in the uterine endometrial cavity in the immunologically deteriorated state of patients. Cancer, for example, uterine endometrial cancer, presents a similar state.

There are various epidemiological risk factors for uterine endometrial cancer \(^1\). It is possible that the bacteria detected in the uterine endometrial cavity produce carcinogens such as N-nitro compounds \(^3\)\(^{-7}\), \(n\)-butyric acid and \(n\)-valeric acid \(^8\)\(^{-13}\) or some growth factors like transforming growth factor, insulin-like growth factor, epidermal growth factor, and membrane-mediated growth factor \(^21\)\(^{-23}\).

Mixed abnormal flora between aerobic and anaerobic bacteria were detected in all patients with uterine endometrial cancer. It is suggested that the lesion of uterine endometrial cancer provides favorable conditions for bacterial growth. Mixed abnormal bacterial flora also might influence the onset and growth of uterine endometrial cancer.

References


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子宮体癌患者における子宮内細菌叢の検討

岐阜大学医学部産科婦人科

三鴨 廣繁 和泉 孝治 伊藤 邦彦 玉倉 輝彦

附属健気性菌実験施設

渡辺 邦友 上野 一 恵

概 要
ある種の細菌はニトリ化合物やn-酪酸や興草酸などを産生する。この観点から子宮体癌患者における子宮内細菌叢を検討することは重要である。

今回我々は、子宮体癌患者20名と子宮筋腫以外に合併症を持たない患者20名の子宮内細菌叢を検討した。
その結果、子宮体癌患者の子宮内では、腸内細菌、Streptococcus agalactiae や嫌気性菌が優位を占めていた。この事実より、これらの細菌の産生物が子宮体癌発生のイニシエーターのひとつになっている可能性がある。

また、今回検討した子宮体癌患者の子宮内には、全例に好気性菌と嫌気性菌の両方が検出された。この事実は、これらの細菌が子宮体癌の発生と進行に影響を及ぼしている可能性がある。