Internal Bacterial Flora of Solid Uterine Cervical Cancer

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

Department of Obstetrics and Gynecology, School of Medicine, Gifu University, Gifu, Japan

*Institute of Anaerobic Bacteriology, School of Medicine, Gifu University, Gifu, Japan

(Received: April 22, 1993)

(Accepted: July 13, 1993)

Key words: uterine cervical cancer, uterine cervical cancer, aerobic bacteria,
anaerobic bacteria

Abstract

Twenty-five patients with uterine cervical cancer (Two cases stage 0; four cases stage Ia; five cases stage Ib; one case stage IIa; ten cases stage IIb; two cases stage IIIa; one case stage IVa) served as the subjects. The bacterial flora inside the cervical cancers was investigated using the optimal technique. There was mixed abnormal aerobic and anaerobic bacterial flora in all subjects.

The average number of bacterial species isolated from inside the cervical cancers was 6.3.

The predominant bacteria isolated were the aerobes, *Staphylococcus haemolyticus*, *Streptococcus agalactiae*, *Escherichia coli* and *Gardnerella vaginalis*, and *Prevotella bivia*. As the stages of cervical cancer progressed, *G. vaginalis*, *B. distasonis* and *P. bivia* were detected at higher rates and higher counts than other bacteria.

These findings suggest that there is a relationship between *G. vaginalis*, *B. distasonis* and *P. bivia* and the onset and growth of cervical cancer.

Introduction

We investigated abnormal bacterial flora inside solid uterine cervical cancers in connection with the onset and growth of the cancer and postoperative antimicrobial chemotherapy. There have been reports of abnormal bacterial flora in the vagina and the cervix in uterine cervical cancer¹⁾, but no investigations of bacterial flora inside uterine cervical cancer had yet been performed.

Accordingly, we investigated the bacterial flora inside solid uterine cervical cancers using the optimal methods.

Materials and Methods

1. Subjects

Twenty-five surgical patients with a diagnosis of stage 0, Ia, Ib, IIa, IIb, IIIa or IVa of uterine cervical cancer were studied. Their age ranged from 38 to 78 years, and the mean age was 58.0 years. All patients underwent hysterectomy in the Department of Obstetrics and Gynecology of the School of Medicine, Gifu University, between June 1990 and December 1992. The histological type of the lesion was squamous cell carcinoma and in 23 cases and adenosquamous cell carcinoma in 2 cases.

2. Collection and processing of specimens

Immediately after total abdominal hysterectomy, samples were taken directly from inside solid uterine cervical cancers using a polyesterfiber 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) 1.0 g, Agar 1.0 g, Distilled water 1000 mL/pH 7.2. All of the components were mixed together, and a solution was prepared by heating at 80°C for 30 minutes. After transferring 9 mL of the buffer to each of the test tubes, the air in the tubes was replaced with CO₂, and the tubes were immediately sealed with a butyl rubber stopper. All tubes and solutions were sterilized in an autoclave at 115°C for 20 minutes.

After suspending the samples, the tubes were re-sealed under a continuous stream of commercial grade of carbon dioxide to drive air out. Exposure of the samples to atmospheric oxygen was restricted to 5 minutes or less. Incubation was commenced immediately after the samples had been suspended in the solution.

Aliquots were aspirated through a butyl rubber stopper using a syringe and retained for subsequent quantitative culture.

The best sampling method is aspiration, but since it is impossible to take aspirated specimens in the case of uterine cervical cancer, we collected the specimens with swabs.

3. Quantitative bacteriologic assay

Serial dilutions of 10², 10⁴, and 10⁶ were prepared in anaerobic buffer. A 0.1 mL sample of each dilution was plated on each of 8 media.

For anaerobic organisms, Brucella HK (Hemin, Vitamin K1), RS (Rabbit, Sheep), blood agar (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan), PEA (β -phenylethylalcohol) laked Brucella HK blood agar (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan), PV (Paromomycin, Vancomycin), Brucella HK blood agar (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan) and Bacteroides bile esculin agar (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan). These anaerobic agar plates were stored under anaerobic conditions prior to use.

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 (Becton Dickinson, Cockeysville, U.S.A.), *Staphylococcus* selective agar (Nissui Pharmaceutical Industrial Co., Tokyo, Japan). After incubation, morphologically distinct colonies on the plates were subcultured onto respective blood agar plates and purified by standard microbiologic methods. Quantitative results are expressed as ranges and mean log₁₀ of viable bacteria per swab.

4. Identification of isolates

All bacterial isolates were identified at the genus and species level. Anaerobic isolates were identified using the Rap ID ANA II identification system (Innovative Diagnostic System, Inc., Atlanta, GA) combined with gas-liquid chromatography (GLC) to identify fatty acids produced during growth in PYG broth (Scott Laboratories, Rockville, MD). Isolates that were difficult to identify were subjected to techniques described in the anaerobic laboratory manual^[2].

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

STREP identification system.

Results

The species of bacteria present in the sampling solution and the case numbers are shown in Table 1.

Table 1 Bacteria detected inside solid uterine cervical cancers

Clinical stage(FIGO)		O		Ia			Ib			IIa		IIb										IIIa		IVa				
Case Number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		
AEROBES	Bacteria																											
	<i>S. epidermidis</i>		++	##	##												+					+				++		
	<i>S. aureus</i>									++	+				+	+	++									++		
	<i>S. haemolyticus</i>					+			++				++					++			+	+			+	+		
	<i>S. hominis</i>		++			++		++										+		##				++				
	<i>S. capitis</i>		+																									
	<i>S. xylosum</i>																		+			++				+		
	GPC	<i>S. auricularis</i>							++												+			+				
		<i>S. sciuri</i>																+										
		<i>S. agalactiae</i>											##		++				++			+	+			##	++	
		<i>S. oralis</i>			+												++									##	++	
		<i>S. intermedius</i>							++											++				++				
		<i>G. morbillorum</i>										+						+										
		<i>E. faecalis</i>									##				##	+				++			++				++	
		<i>E. faecium</i>				##								++						++							+	
		<i>Corynebacterium</i> spp.		++						++					+													
	CPB	GPB															++											
		<i>G. vaginalis</i>		##		##	##	##	##							++	##		##	++	+		##	##				
		<i>Lactobacillus</i> spp.		++				++	++		++			+	++		+				++				++			
	GNB	<i>E. cloacae</i>												++														
		<i>E. coli</i>			++	++		##		++		++	++	++	++	+				+	+		±	++			+	
	ANAEROBES	<i>P. anaerobius</i>																++				+					++	
		<i>P. asaccharolyticus</i>												++					++									
		GPC	<i>P. magnus</i>			+		+							++													
			<i>P. micros</i>		++			++		##		++			+	+			++						++			
<i>P. prevotii</i>											+						++											
<i>P. tetradius</i>																				+					++			
<i>Bifidobacterium</i> spp.						++						+												++				
GPB		<i>C. innocuum</i>																					+					
		<i>P. acnes</i>		++	+																				##	+		
		<i>B. fragilis</i>					++		##									+			+					##		
GNB		<i>B. distasonis</i>								##	++	##	##	##	##	##	##	++				##	++		##			
		<i>B. ovatus</i>												+	+													
		<i>B. thetaiotaomicron</i>						##													##		++					
		<i>P. bivia</i>														++	++		++			++	++				##	
		<i>P. disiens</i>				++													++				+				+	
	<i>P. oralis</i>																+						+					
	<i>P. intermedia</i>																		++									
	<i>Capnocytophaga</i> sp.																							++				
	<i>Wolinella</i> spp.								+													++						
Total		4	4	6	5	5	5	5	5	5	4	5	10	7	8	7	7	6	6	7	7	9	9	8	8	6		

(10² < -, 10² < + < 10⁴, 10⁴ < ++ < 10⁶, 10⁶ < +++ < 10⁸, 10⁸ < ####)

FIGO: International Federation of Gynecology and Obstetrics

Both aerobic and anaerobic bacteria were detected in all subjects.

The average number of species isolated from inside solid uterine cervical cancers was 6.3.

The following aerobic bacteria were detected: *Staphylococcus epidermidis* in six cases (24.0%), *Staphylococcus aureus* in six cases (24.0%), *Staphylococcus haemolyticus* in eight cases (32.0%), *Staphylococcus hominis* in six cases (24.0%), *Staphylococcus capitis* in one case (4.0%), *Staphylococcus xylosus* in three cases (12.0%), *Staphylococcus auricularis* in three cases (12.0%), *Staphylococcus sciuri* in one case (4.0%), *Streptococcus agalactiae* in seven cases (28.0%), *Streptococcus oralis* in two cases (8.0%), *Streptococcus intermedius* in three cases (12.0%), *Gemella morbillorum* in two cases (8.0%), *Enterococcus faecalis* in six cases (24.0%), *Enterococcus faecium* in four cases (16.0%), *Corynebacterium* spp. in three cases (12.0%), *G. vaginalis* in 11 cases (44.0%), *Lactobacillus* spp. in eight cases (32.0%), a Gram-positive bacillus in one case (4.0%), *Enterobacter cloacae* in one case (4.0%) and *Escherichia coli* in 13 cases (52.0%).

Among these aerobic bacteria, the isolation rates for *S. haemolyticus*, *S. agalactiae*, *G. vaginalis*, *Lactobacillus* spp., *E. coli* was rather high (greater than 25%). The bacterial counts of *G. vaginalis*, *S. agalactiae* and *E. coli* were greater than those of other species.

The following anaerobic bacteria were detected: *Peptostreptococcus anaerobius* in three cases (12.0%), *Peptostreptococcus asaccharolyticus* in two cases (8.0%), *Peptostreptococcus magnus* in three cases (12.0%), *Peptostreptococcus micros* in eight cases (32.0%), *Peptostreptococcus prevotii* in two cases (8.0%), *Peptostreptococcus tetradius* in two cases (8.0%), *Bifidobacterium* spp. in three cases (12.0%), *Clostridium innocuum* in one case (4.0%), *Propionibacterium acnes* in four cases (16.0%), *Bacteroides fragilis* in five cases (20.0%), *Bacteroides distasonis* in nine cases (36.0%), *Bacteroides ovatus* in two cases (8.0%), *Bacteroides thetaiotaomicron* in three cases (12.0%), *Prevotella bivia* in six cases (24.0%), *Prevotella disiens* in four cases (16.0%), *Prevotella oralis* in two cases (8.0%), *Prevotella intermedia* in one case (4.0%), *Capnocytophaga* sp. in one case (4.0%) and *Wolinella* spp. in two cases (8.0%).

All 25 subjects had one or more species of anaerobic bacteria. Five species of bacteria were detected in two cases, four species in three cases, three species in six cases, two species in nine cases, and only one species in five cases. Both aerobic and anaerobic bacteria were detected in every case.

P. micros, *B. distasonis* and *P. bivia* were detected more often than other anaerobic bacteria.

Discussion

The bacterial flora in the genital tract of normal women are thought to be influenced by various factors, such as hormonal factors, aging, etc. There was little relationship between the bacterial flora of cervical cancer and patient age, suggesting that estrogen did not have much effect.

As the stage of cervical cancer became more advanced, *S. agalactiae*, *E. coli*, *G. vaginalis*, *B. distasonis* and *P. bivia* were detected at higher counts than other bacteria detected from uterine cervical cancers.

Bacteria detected inside solid uterine cervical cancers may produce carcinogens or growth factors^{4,5,6,7,8,9,10}.

Mixed aerobic and anaerobic abnormal bacterial flora were detected in all of the patients with uterine cervical cancer. It appears that the presence of uterine cervical cancer affected the growth of the bacteria, or that mixed abnormal bacterial flora influenced the onset and growth of uterine cervical cancer.

References

- 1) Ito, K., Tamaya, T., Watanabe, K. & Ueno, K.: Intravaginal bacterial flora in patients with cervical cancer especially *Gardnerella vaginalis*. Acta Obst Gynaec Jpn 42(11): 1551—1555, 1990.
- 2) Holdeman, L.V.E.P., Cato, E.P. & Moore, W.E.C.: Anaerobe laboratory manual. 4th edition. Anaerobe Laboratory, Virginia, 1977.
- 3) Ralt, D., Wishnok, J.S., Fitts, R. & Tannebaum, S.R.: Bacterial catalysis of nitrosation: Involvement of *nar* operon of

- Escherichia coli*. J Bacteriol, 359—364, 1988.
- 4) Calmels, S., Ohshima, H., Vincent, P., Gounot, A.M. & Bartsch, H.: Screening of microorganisms for kinetic studies on nitrosamine formation from secondary amines by *E. coli* strains. Carcinogenesis, 6(6): 911—915, 1985.
 - 5) Calmels, S., Ohshima, H., Rosenkrantz, H., MacCoy, E. & Bartsch, H.: Biochemical studies on the catalysis of nitrosation by bacteria. Carcinogenesis, 8(8): 1085—1088, 1987.
 - 6) Pavic, N.: Is there a local production of nitrosamines by the vaginal microflora in anaerobic vaginosis/trichomoniasis?. Med Hypoth, 15: 433—436, 1984.
 - 7) Kishishita, M., Yanase, S. & Ito, Y.: Activation of Epstein-Barr virus expression in human lymphoblastoid P3HR-1 and Raji cells with propionic acid and with culture fluids of propionic acid-producing anaerobes. Cancer Lett, 16: 117—120, 1982.
 - 8) Leder, A. & Leder, P.: Butyric acid, a potent inducer of erythroid differentiation in cultured erythroleucemic cells. Cells, 5: 319, 1975.
 - 9) Hill, J.M.: Bacteria and human carcinogenesis. Bacteria and cancer, Academic Press, 27—47, 1982.
 - 10) Imai, A., Matsunami, K., Ohno, T. & Tamaya, T.: Enhancement of growth-promoting activity in extract from uterine cancers by protein kinase-C in human endometrial fibroblasts. Gynecol Obstet Invest, 33: 109—113, 1992.

子宮頸癌固形腫瘍内部の細菌学的検討

岐阜大学医学部産科婦人科, *同 附属嫌気性菌実験施設

三鴨 廣繁 和泉 孝治 伊藤 邦彦
渡辺 邦友* 上野 一恵* 玉舎 輝彦

(平成 5 年 4 月 22 日 受付)

(平成 5 年 7 月 13 日 受理)

子宮頸癌患者 25 名 (0 期 2 例, Ia 期 4 例, Ib 期 5 例, IIa 期 1 例, IIb 期 10 例, IIIa 期 2 例, IVa 期 1 例) の固形腫瘍内部の細菌学的検討を行った。全症例で、好気性菌と嫌気性菌が混在していた。1 症例あたりの平均分離株数は、6.3 株であった。

分離された細菌は、好気性菌では、*Staphylococcus haemolyticus*, *Streptococcus agalactiae*, *Escherichia coli*, *Gardnerella vaginalis* が、嫌気性

菌では、*Peptostreptococcus micros*, *Bacteroides distasonis*, *Prevotella bivia* が優位を占めていた。特に、子宮頸癌進行期の進展とともに、*G. vaginalis*, *B. distasonis*, *P. bivia* などの細菌が他の細菌に比較して高率に検出された。

以上より、*G. vaginalis*, *B. distasonis*, *P. bivia* などの細菌が、子宮頸癌の発生や進行に関与している可能性が示唆された。