

Multinational Comparison of Diagnostic Clues for Uterine Cervical Lesions Among Cytotechnologists in Asian Countries

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Vaccination has been underway in several countries for sexually inactive young girls or women against HPV 16 and 18 to prevent them from infection of these HPV types and concurrent cancer development. However, uterine cervical cancers may remain uncontrolled among some Asian countries, where other types of HPV infection are more frequent. A sensitive cancer screening system would remain important for detection of the earlier stage cervical cancers in Asian countries.

In this study, 130 cytotechnologists (CTs) in Asian countries (Taiwan 80, Japan 18, Korea 15, Thailand 11, Singapore 3, Bhutan 2, and Mongolia 1) participated in the vote. Selected 10 cervical Pap smears that would be adequate to identify the diag-

nostic clues especially for atypical squamous cells (ASC) with two or three representative pictures for each case were displayed on the website. The percentages of consistent diagnosis voted by certified CTs with ≥ 5 years of experience were compared among 10 cervical cases or among Asian countries enrolled. As results, low consistency for ASC cases and high consistency for squamous intraepithelial lesion (SIL) were observed. Examining specimens for the diagnostic clues of ASC in TBS is crucial to maintain the high sensitivity and positive predictive value of SIL in Asian countries. Diagn. Cytopathol. 2010;00:000–000. © 2010 Wiley-Liss, Inc.

Key Words: liquid-based cytology; The Bethesda system; uterine cervix; human papillomavirus; vote

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It has been well known that uterine cervical cancer can develop after the incorporation of particular types of human papillomavirus (HPV) into the basal cells of uterine cervical squamocolumnar junction.¹ Vaccination has been underway in several countries for sexually inactive young girls or women against HPV 16 and 18 to prevent them from infection of these HPV types and concurrent cancer development. About 70% of vaccinated female would be successfully protected from infection of these HPV types if they were between 12 and 15 years old at vaccination.² This optimistic outcome, however, possibly may be restricted to countries where HPV 16 and 18 are most prevalent.³ Among some Asian countries, where other types of HPV infection are more frequent, uterine cervical cancers may remain uncontrolled³ although cross protection by vaccination against HPV 45 could be expected in parts.⁴ A sensitive cancer screening system

would remain important for detection of the earlier stage cervical cancers in Asian countries.

The authors discussed the method of cervical cancer screenings and HPV prevalence in Asian countries at the 15th Japan-Thailand International Cytology Workshop (JTICW) held in Tokyo in December 2008. Prior to this meeting, K.T. and H.K. had organized an international vote on smears from cervical lesions by cytotechnologists (CTs) in Asian countries via the internet to compare their diagnostic clues for cervical lesions. We found differences and similarities in the diagnostic clues by CTs in Asian countries. CTs would play an important role in managing an effective screening system to protect patients from developing advanced fatal cervical diseases caused by other types of HPV in Asian countries. In this study, we have highlighted the characteristics of Japanese CTs.

Materials and Methods

Assessment of Cervical Lesions by CTs

A selected assessment on 10 cervical lesions was performed approximately 20 days before the 15th JTICW. K.T. asked co-authors to introduce this vote to CTs in their own countries, and also asked N.C.J. and D.N.J. to prepare 10 cervical smears obtained from patients in the US that would be adequate to identify the diagnostic clues especially for atypical squamous cells (ASC). These cases were from archives and patients had given their consent at the time. H.K. designed the website with help from the Japanese Society of Clinical Cytology (JSCC). CTs who participated in this vote were asked to register their biographies including their names as well as responses (Table I). CT names were requested to avoid double answers and H.K. kept the names confidential. On the website, 10 cases with two or three representative pictures for each case were displayed (Fig. 1). Patient's age, clinical information, past history, and preparation method of smears were given at the beginning of each case presentation. The cytological diagnoses made by N.C.J and D.N.J were not revealed until end of voting period even to K.T. and H.K. (Table II).

Statistical Analysis

All data obtained by vote were stored by H.K. and analyzed by K.T. Answers were compared with those prepared by N.C.J and D.N.J. and an identical answer was counted as a consistent diagnosis. The percentages of consistent diagnosis voted by CTs were compared among 10 cervical cases or among Asian countries enrolled. When the $P < 0.05$, it was considered as statistically significant.

Results

Summary of Cytotechnologist Data

In total, 130 cytotechnologist (CTs) were enrolled in this study and they consisted of 80 members from Taiwan, 18

Table I. Vote on 10 Cervical Pap Smear Cases

I Categories of diagnosis
a) NILM, b)ASC-US, c) LSIL, d) ASC-H, e) HSIL, f) FSFI, g) SCC, h) AGC (NOS), i) AGC favor neoplastic, j) AIS, k) ADC
II Biography
1) Nationality: (j) Japan, (k) Korea, (s) Singapore, (ta) Taiwan, (th) Thailand, or (o) others
2) Gender: (m) male, or (f) female
III Experience as an authorized Cytotechnologist
(T) trainee, (<1) less than one year, (<5) less than 5 year, or (5) five years or more
IV Specialty
(Gy) Gynecological, (Ge) General, or (OT) Others
V Usual way of cervical cytology reporting
1) The Bethesda System: (y) yes or (n) no
2) Liquid-Based Cytology: (y) yes or (n) no

NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; AGC, atypical glandular cells; NOS, not otherwise specified; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; FSFI, HSIL with features suspicious for invasion; AIS, adenocarcinoma in-situ; SCC, squamous cell carcinoma; ADC, adenocarcinoma.

from Japan, 15 from Korea, 11 from Thailand, and 6 from other countries (Singapore 3, Bhutan 2, and Mongolia 1). CTs were comprised of 32 males and 94 females. Cytological diagnoses by well-experienced CTs are usually more complete and consistent than that observed for less-experienced CTs. CTs that had ≥ 5 years of experience totaled 106 (Japan 15, Korea 14, Thailand 10, Taiwan 64, and others 3) with 26 males and 80 females. Of these, 44 (41.5%), 3 (2.8%) and 59 (55.7%) listed gynecology, others and general fields for their specialty, respectively. For routine work in cytopathology, 89 (84.0%) and 39 (36.8%) used the Bethesda system (TBS) and liquid based cytology (LBC) preparation, respectively. No gynecology specialist from Japan was enrolled in this study, and just a few CTs used TBS for routine work. Except for Korea, LBC was not the preferred method in participating Asian countries (Table III).

Table IV summarizes consistent diagnoses voted by participating certified CTs with experience of ≥ 5 years. Consistency less than 50% was observed for Cases 1 (ASC- of undetermined significance: US), 3 (ASC- cannot exclude high-grade squamous intraepithelial lesion: H), 2, 9, 10 (negative for intraepithelial lesion or malignancy: NILM). Among the enrolled Asian countries, the lowest and highest consistencies were observed in Japan and Thailand, respectively.

Japanese CTs With Five or More Years of Experience

To highlight the characteristics of Japanese CTs, diagnoses voted by the 15 Japanese CTs with ≥ 5 years of experience were compared with the performance of other specified groups, including 38 CTs of similar length of experience whose specialty was gynecology using TBS for routine work (Gy + TBS), and 46 CTs of similar

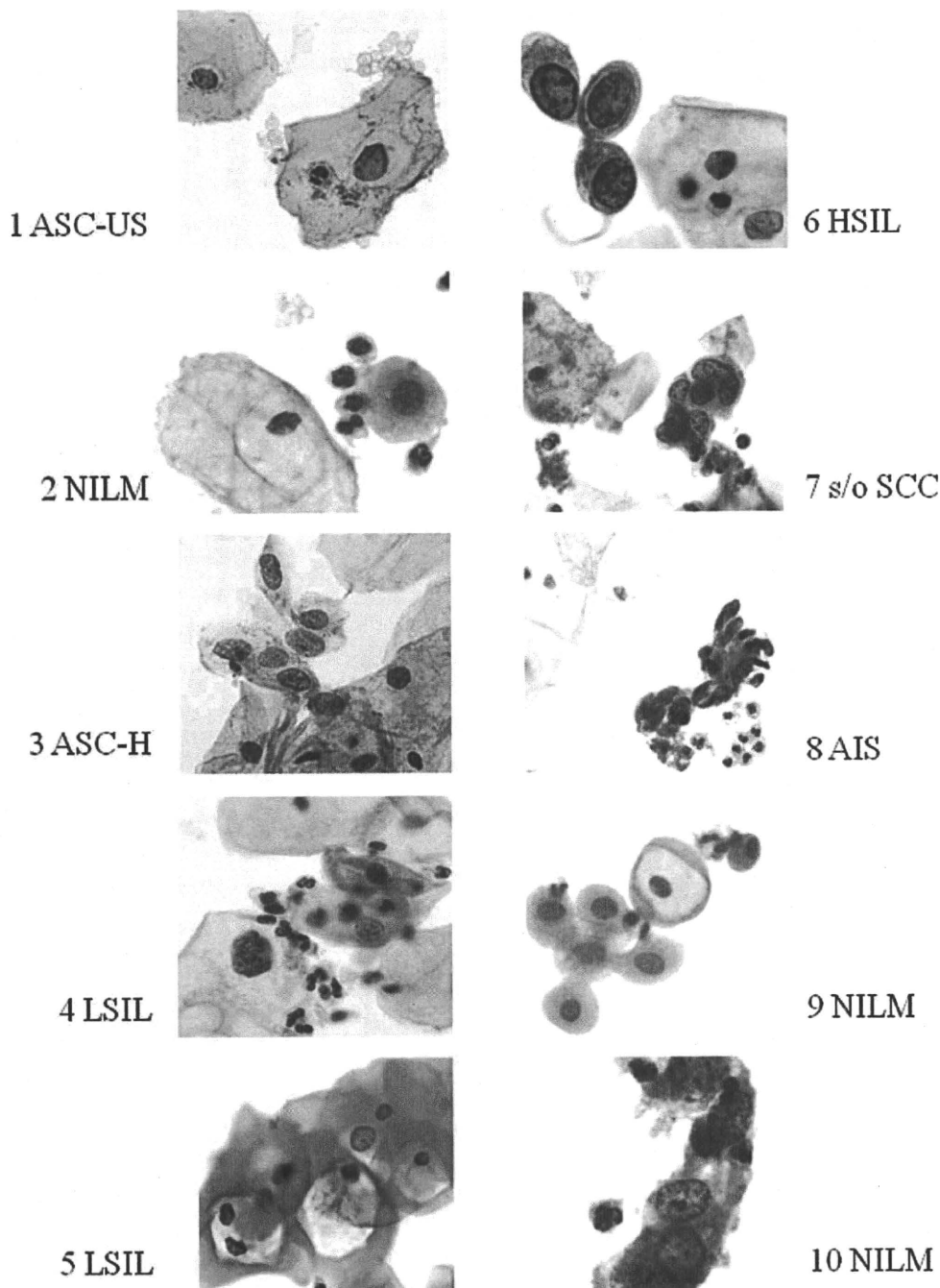


Fig. 1. Representative pictures of 10 cervical Papanicolaou smear cases with an original objective magnification of 40× for an internet voting. Every Papanicolaou smears were prepared with liquid-based cytology method and Papanicolaou stained. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

length of experience whose specialty was others or general field (Ge + OT). The averages of consistent diagnoses for 10 cervical cases were 40.0% for Japanese CTs, 47.2% for the 38 Gy + TBS CTs and 49.1% for the 46 Ge + OT CTs. Japanese CTs showed the highest frequency of consistent diagnosis in low-grade squamous intraepithelial lesion (LSIL) (Case 4) and high-grade squamous intraepithelial lesion (HSIL) (Case 6), and the

lowest frequency of consistent diagnoses in NILM (Cases 2 and 10), ASC-H (Case 3), LSIL (Case 5), HSIL (Case 7), and adenocarcinoma in situ (AIS) (Case 8) as shown in Table V and Figure 2.

Discussion

HPV vaccination has been underway in several countries. Currently, two different vaccines, Merck (Gardasil[®]) and

Table II. List of 10 Cervical Pap Smear Cases

Case	Age	Clinical information or past history	Preparatoin	Pictures no.	Cytological dx
1	40	Routine screening	LBC	3	ASC-US
2	20	Patient on Depo Provera, Prior 2 smears (2 years); LSIL with HPV	LBC	3	NILM
3	47	Abnormal smear, Carcinoma in-situ of the cervix	LBC	2	ASC-H
4	33	On Birth control pills, moderate dysplasia	LBC	3	LSIL
5	47	Routine screening	LBC	3	LSIL
6	33	Routine screening	LBC	3	HSIL
7	26	Mild dysplasia	LBC	3	S/O SCC (HSIL)
8	38	Routine screening	LBC	3	AIS
9	23	ASC-US twice, LSIL with HPV once	LBC	3	NILM
10	20	Routine screening	LBC	3	NILM

NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; AIS, adenocarcinoma in-situ; s/o, suspicious of; SCC, squamous cell carcinoma.

Table III. Certified Cytotechnologists With Experience of Five or More Years Participated

Nations	Number	Gender		Specialty				Method				
		Male/Female	Gynecology	General	Others	TBS	LBC					
Japan	15	7/8	0	15	100%	0	3	20.0%	6	40.0%		
Korea	14	6/8	6	42.9%	8	57.1%	0	14	100%	2	20.0%	
Thailand	10	5/5	2	20.0%	8	80.0%	0	10	100%	2	20.0%	
Taiwan	64	8/56	34	53.1%	27	42.2%	3	4.7%	59	92.2%	16	25.0%
Others	3	0/3	2	66.7%	1	33.3%	0	3	100%	2	66.7%	
Total	106	26/80	44	41.5%	59	55.7%	3	2.8%	89	84.0%	39	36.8%

TBS, the Bethesda system; LBC, liquid based cytology.

Table IV. Consistent Diagnoses by Cytotechnologists With Experience of Five or More Years Participated

Nations	Number	Percentages of diagnoses voted										Average
		Case 1 ASC-US	Case 2 NILM	Case 3 ASC-H	Case 4 LSIL	Case 5 LSIL	Case 6 HSIL	Case 7 HSIL	Case 8 AIS	Case 9 NILM	Case 10 NILM	
Japan	15	20	6.7	26.7	80	73.3	93.3	40	46.7	13.3	0	40.0
Korea	14	50	7.1	64.3	57.1	64.3	78.6	71.4	14.3	7.1	14.3	42.9
Thailand	10	60	60	80	90	100	90	90	40	30	40	68.0
Taiwan	64	17.2	26.6	42.2	65.6	90.6	81.3	60.9	71.9	10.9	7.8	47.5
Others	3	0	33.3	0	33.3	100	66.7	60.7	0	33.3	33.3	36.1
Total	106	25.5	24.5	45.3	67.9	85.8	83.0	62.3	55.7	13.2	11.3	47.5

NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; AIS, adenocarcinoma in-situ.

Table V. Comparison of Consistent Diagnoses by Japanese and Other Cytotechnologists With Experience of Five or More Years Participated

Cytotechnologists	Number	Percentages of diagnoses voted										Average
		Case 1 ASC-US	Case 2 NILM	Case 3 ASC-H	Case 4 LSIL	Case 5 LSIL	Case 6 HSIL	Case 7 HSIL	Case 8 AIS	Case 9 NILM	Case 10 NILM	
Japanese	15	20	6.7	26.7	80	73.3	93.3	40	46.7	13.3	0	40.0
Gy + TBS	38	16.4	31.6	44.7	57.9	92.1	71.1	63.2	57.9	23.7	13.2	47.2
Ge + OT	46	30.4	26.1	47.8	69.6	87	87	69.6	52.2	6.5	15.2	49.1

NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; AIS, adenocarcinoma in-situ; Gy, gynecology; TBS, the Bethesda system; Ge, general; OT, others.

GSK (Cervarix®), are available commercially. These vaccines have been reported to be responsible for ~70% of cervical cancer following HPV 16 and 18 infections.² Although cross protection against HPV Types 45, 33, and

31 has been reported,^{4,5} other types of HPV do not have a vaccination and they are more prevalent in Asia than that in the West. In Japan, HPV 16 and 18 were identified in 47% of cervical cancers, which was much lower than the

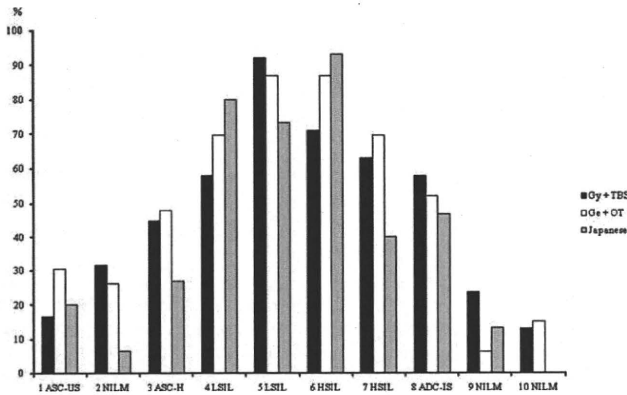


Fig. 2. Concordance rates of answers in each case voted by certified cytothechnologists with ≥ 5 years of experience. NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; AIS, adenocarcinoma in situ; Gy, gynecology; TBS, the Bethesda system; Ge, general; OT, others. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Western prevalence of 70%. HPV 52 was the third most frequent type in cervical cancer cases, and the second most frequent type in HSIL cases, in which HPV 58 was the third most frequent type.⁶ HPV 58 is common in cervical cancer as well as in the general population throughout Asia. HPV 51, 52, and 56 also appeared to be relatively more common, while types 31, 33, and 35 were much less common than in the West.⁷ Recently, some HPV types, such as HPV 52, have shown that they may not be amplified by GP5+/6+ polymerase chain reaction (PCR) as effectively as HPV 16 and 18 because of sequence mismatches between the target gene and the primers. The prevalence of HPV 52 infection may have been underestimated in previous studies. In the Philippines, HPV 52 is the most common as determined by PCR using modified GP5+/6+ primers among 56 sex workers with abnormal cervical cytology (LSIL, HSIL, and AIS). To contrast, the most common HPV type in women with squamous cell carcinoma by PCR using the original GP5+/6+ primers was reported to be HPV 16, followed by HPV 18.³ These findings may indicate that the HPV 52 could be more prevalent than what has been reported literally in Asia, and the vaccination against HPV 16 and 18 would be less effective in Asia than that expected in the West. Therefore, cervical cancer would still remain as one of the leading causes of death for women even after the two types of vaccinations have been given. Asian countries must collaborate to share a strategy to treat patients infected with those HPV types since a vaccine is unavailable.

TBS and LBC preparation were originally developed in the USA to replace the Papanicolaou classification and conventional Pap smears of cervical cytology.⁸ Currently,

TBS is a common classification for cervical cytology in Asia except for Japan but LBC is not a common procedure in Asia (Table III). The introduction of TBS in 1988 eliminated the diagnostic category of moderate dysplasia or cervical intraepithelial neoplasia (CIN) 2 in three categories (CIN 1, 2, 3), which expressed the cellular changes caused by HPV infection, and proposed a bipartite division, LSIL and HSIL in the US. Revisions to TBS occurred in 1991 and 2001. In the 1991 Bethesda classification, "Favor reactive" and "Not otherwise specified" were used in the ASC category, but were eliminated in 2001. Currently, ASC is divided into ASC-US and ASC-H, and atypical glandular cells are grouped into AGC.

ASC refers to cytological changes suggestive of SIL, which are qualitatively or quantitatively insufficient for a definitive interpretation. According to the criteria of ASC-US in the 2001 TBS, nuclei are approximately two and one half to three times the area of the nucleus of a normal intermediate squamous cell with slightly increased ratio of nuclear to cytoplasmic area (N/C) and minimal nuclear abnormality such as hyperchromasia and irregularity in chromatin distribution or nuclear shape. ASC-H cells are defined as those occurring singly or in small fragments of less than 10 cells with N/C ratio approximately to that of HSIL. They are also referred to as atypical (immature) metaplasia. Most ASC-US interpretations are suggestive of LSIL, but 10% to 20% have an underlying CIN 2 or CIN 3. ASC-H is expected to be associated with a higher positive predictive value for detecting an underlying CIN 2 or CIN 3 than ASC-US, but is less predictive of CIN 2 or worse than definitive interpretations of HSIL.⁹

In the present study, a high concordance rate was observed in more than 50% of the answers in the cases of LSIL, HSIL and AIS. However, the concordance rates in the cases of NILM, ASC-US and ASC-H were less than 50%. ASC does not indicate the special findings of individual cells because it needs the entire specimen for interpretation. Due to the limited number of pictures displayed on the website voting, ASC and NILM were difficult in the present study. Even in the US, the concordance rate of ASC is low as indicated in TBS website atlas of the American Society of Cytopathology (<http://nih.techriver.net/>). In four ASC-US cases in this atlas, the consistent interpretation was ranged from 35% to 47%. On the other hand, SIL and AIS can be diagnosed when their findings appeared on the pictures viewed. The participants in the present study demonstrated a good ability to detect the findings of SIL and AIS in the pictures viewed (Table IV).

Among well-trained CTs with an experience as certified CTs for ≥ 5 years in four Asian countries (Japan, Korea, Thailand, Taiwan), Japanese CTs voted the lowest and Thai CTs voted the highest consistently for diagnoses of 10 cervical lesions. This difference might be relating partly to the familiarity of CTs participated with TBS

since only 20% of participated Japanese CTs used TBS for their routine work. In Japan, a modified TBS for uterine cervical lesions was introduced officially in 2009. The majority of Japanese CTs still use the Papanicolaou classification and LBC preparation has been introduced into a small number of laboratories, so far. However, they maintain an accurate level of diagnosis by passing the examination organized by JSCC and attending continuing education courses related to diagnostic cytopathology or JSCC annual meeting. When comparing the certified Japanese CTs with other specified two groups (Gy + TBS, Ge +OT), the certified Japanese CTs voted LSIL in Case 4 and HSIL in Case 6 more frequently but least frequently for ASC-US in Case 1, and ASC-H in Case 3 as shown Figure 2. These results suggest that Japanese CTs who have been well trained to diagnose all CIN 1, 2, and 3 correctly can adapt to TBS and LBC in cases of SIL well, but not in cases of ASC even though ASC was difficult to be selected correctly on the website voting.

The combination of HPV genotyping and cytological testing are vital to achieving an effective cancer screening system and an earlier detection of CIN 3 lesions.¹⁰ Asian countries share the same problem in that some prevalent HPV types have no available vaccine. It is preferable that CTs among Asian countries have the same diagnostic clues for uterine cervical lesions. However, as indicated in this study, CTs among Asian countries have different familiarity with TBS and LBC, and their consistency to diagnose ASC was very low. As it has been reported that examining the specimens for the diagnostic clues for ASC is crucial to maintain the high sensitivity and positive predictive value of SIL,⁹ a multinational comparison of diagnostic clues for uterine cervical lesions among Asian countries should be important to treat the patients under a

same strategy in Asian countries. An international comparison for the diagnostic clues of uterine cervical lesions has been undertaken for the first time among Asian countries up to our knowledge.

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SHORT REPORT

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Japanese experience of hydrogen sulfide: the suicide craze in 2008

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Abstract

Most of hydrogen sulfide poisoning has been reported as industrial accidents in Japan. However, since January 2008, a burgeoning of suicide attempts using homemade hydrogen sulfide gas has become evident. By April 2008, the fad escalated into a chain reaction nationwide. Mortality of the poisoning was very high. There were 220 cases of attempted gas suicides during the period of March 27 to June 15, killing 208. An introduction of new method of making the gas, transmitted through message boards on the internet, was blamed for this "outbreak". The new method entailed mixing bath additive and toilet detergent. The National Police Agency instructed internet providers to remove information that could be harmful. Of the victims of the fad in 2008, several cases were serious enough that family members were involved and died. Paramedics and caregivers were also injured secondarily by the gas. This fad has rapidly spread by internet communication, and can happen anywhere in the world.

Overview

Hydrogen sulfide poisoning has been a relatively uncommon intoxication, with only a few cases a year being reported in Japan. Most incidents occurred in circumstances of volcano climbing, pharmaceutical product treatments, and man-hole cleaning[1]. Hence, this poisoning has been categorized as being associated with industrial accidents. However, since January 2008, there has been a burgeoning of suicide attempts using homemade hydrogen sulfide gas. By April 2008, the fad escalated into a chain reaction, and cases of H₂S poisoning made headlines almost everyday, nationwide. The Japanese Cabinet Office reported 220 cases of attempted gas suicides during the period from March 27 to June 15, killing 208, a very high mortality rate (Figure 1). An introduction of new methods of making the gas, transmitted through message boards on the internet, was blamed for this "outbreak." The new method entailed mixing bath additive and toilet detergent. The main component of the bath additive is lime sulfur, and toilet detergent acts as an oxidant to produce H₂S gas. In Japan, the custom of bathing, especially in hot springs (*onsen*), is quite common. As a result, people want to enjoy it in their own homes by using bath additive.

These two materials are thus easily available in Japan, and also obtainable through the internet. Given these circumstances, the National Police Agency instructed internet providers to remove information that could be harmful, and MUTOHAP (the most frequently 'featured' brand of bath additives in the method) was forced to suspend its production. A few cases of swallowing MUTOHAP itself had already been reported as a means of suicide. If the sulfur in MUTOHAP were mixed with gastric acid in the stomach, a H₂S gas-evolving reaction would occur and cause poisoning. When sulfur is mixed with a potent oxidant such as toilet detergent, an even greater quantity of H₂S gas evolves than it would with gastric acid. In most of the cases, victims lose consciousness with a single intake of breath, and die immediately. This has been referred to as *knock down* and was introduced as a painless way to kill oneself.

This new method was first reported in 2007. Because of the burst of gas production in the reaction, it may involve passersby and rescue personnel, not just the person attempting suicide. Of the victims of the fad in 2008, several cases were serious enough that family members trying to rescue their sons or daughters were directly affected and died. In cases where the suicide attempt occurred in a hotel, guests were evacuated[2]. Because of its high water solubility, evaporated gas from the wet clothes of patients can cause secondary poisoning to paramedics and caregivers, too.

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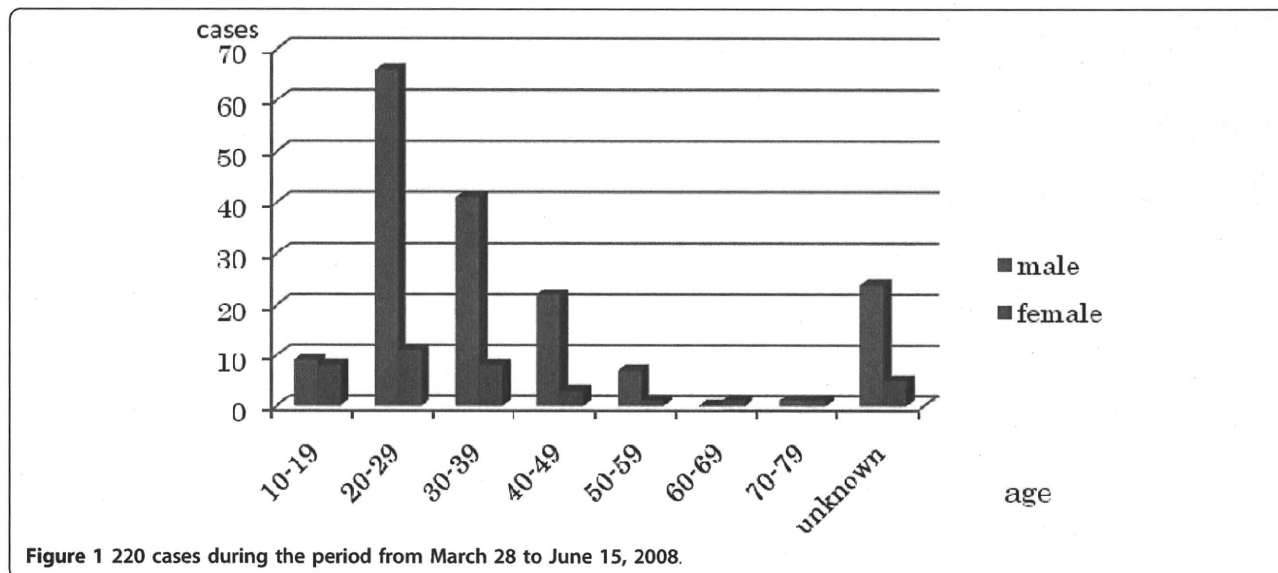


Figure 1 220 cases during the period from March 28 to June 15, 2008.

Profile of hydrogen sulfide

Hydrogen sulfide is a colorless, hydrosoluble and toxic gas with a “rotten egg” smell. This gas is also flammable and can be volatile. It is pungent, often described as “rotten egg”, even at concentrations as low as 0.05 ppm. At higher levels of exposure, a sweet odor can be sensed. Above 100 ppm, its warning odor is said to be lost, because of olfactory nerve paralysis (Table 1). The Japanese Society for Occupational Health sets 10 ppm as the maximum allowable concentration. Its gas specific gravity is 1.188 (comparable to air at 125°C and 1 atmosphere), meaning it is heavier than air. This is one reason why this gas is often associated with accidents in the sewer and mining industry. The gas is not only soluble in water, but also in petroleum.

H₂S inhibits enzymes in mitochondria by binding with Fe³⁺ of cytochrome oxidase. This reaction blocks cellular respiration, and interferes with oxygen utilization at the cellular level. Cyanogen compounds act the same way, and the toxicity is similar. Treatment for H₂S

poisoning is similar to that for cyanogen compounds, as described below.

Specific treatment

Nitrite salt may be efficacious. Nitrite salt oxidizes the Fe²⁺ of hemoglobin (Hb) to Fe³⁺, deriving Met-Hb, which competes with the Fe³⁺ of cytochrome oxidase and protects it from oxidation by sulfide. This mechanism is expected to ameliorate cellular anoxic conditions (Table 2).

The efficacy and administration method of this drug have been discussed in some Japanese language articles. Here is a brief review of those findings[3]. The level of Met-Hb should be monitored when nitrite salt is used as a treatment for H₂S poisoning. Although some experts say that the target Met-Hb level is approximately 30%, it seems feasible to keep the Met-Hb level under 25% with a concern of hypoxemia from methemoglobinemia. One anecdotal report described a case in which the patient was successfully saved with a

Table 1 Effects of H₂S at various concentrations

Concentration of H ₂ S, ppm	Symptoms of exposure
0.05	Pungent smell mimicking “rotten egg”
0.1	Anosmia
50-150	Becoming paralyzed in a few minutes
250	Photophobia, lacrimation, rhinorrhea, cyanosis, pulmonary edema
250-500	Headache, nausea, vomiting, diarrhea, dizziness, palpitation, tachycardia, hypotension, muscle fasciculation, muscle weakness, apnea, disorientation, coma
500-750	Respiratory arrest within 30 to 60 min
750-1000	Collapsing momentarily or knocked down
>1000	Dying immediately within a breath

Table 2 Treatment of H₂S gas poisoning

Amyl nitrite	#. If spontaneous breathing remains, encourage amyl nitrite inhalation from the nasal airway tract. #. Until sodium nitrite is ready, repeat inhalation every 2 to 3 min.
Sodium nitrite	#. Dissolve 0.6 g sodium nitrite to 20 ml of distilled water for injection to make a 3% solution. #. Intravenously administer 10 ml (for child, 0.12-0.33 ml/kg) of the 3% sodium nitrite solution over 20 min or longer. #. Sodium nitrite is not on the market as a medicine, therefore, it requires preparation in each hospital using reagent sodium nitrite. #. Sodium thiosulfate is not efficacious, though it is used to treat cyanogen poisoning. (sodium thiosulfate does not have any negative effect for treatment of H ₂ S poisoning.)

maximum Met-Hb level as low as 14%. Although early administration of this treatment is desirable, there have been cases of both mortality and survival even after patients had entered a state of shock. Another anecdotal study reports that a patient survived without converting hemoglobin to methemoglobin by nitrite salt. However, the severity of those reported cases is assumed to vary, and the method of drug administration is not well established. There is insufficient data to support the widespread use of nitrite salt for H₂S poisoning.

Special concern for secondary disasters

Stirring bath additive and toilet detergent produces a great quantity of lethal gas, more than what is required for an individual suicide from H₂S. Therefore, this can be deleterious for neighbors and rescuers. In the unfortunate fad of 2008, several families of people who attempted suicide became victims themselves. Paramedics and caregivers were also reported to have become injured secondarily. The Tokyo Fire Department alerted family members, neighbors, and hotel staff not to enter any rooms where H₂S was suspected to have been made. Closed rooms or cars proved to be extremely dangerous to enter in an attempt to save loved ones or customers before paramedics arrived.

For paramedics and caregivers, management of a C disaster based on the NBC (Nuclear, Biological and Chemical) disaster is sometimes necessary. After a patient is evacuated, first-step procedures or treatments should be performed in an airy space. Undressing, dry decontamination, is undoubtedly necessary, and if discolored skin is evident, water decontamination such as showering should also be considered. Because H₂S gas is detected in patient expiration, mouth-to-mouth resuscitation is not indicated. An ambulance is a small, enclosed space, so exhaled H₂S gas from a patient can potentially cause poisoning of paramedics. When transferring a patient with H₂S poisoning, all windows should be opened and the vehicle should be well ventilated. Accurate decontamination in the field and in-car ventilation are the most important things to keep paramedics safe from secondary injury. In the same way, caregivers should treat and

decontaminate patients outside of the hospital, behind partitions, for example. However, in most of the cases of H₂S suicide, the victim is the only person to treat. Considering the time it takes to set up a partition, it is not clear how far we should proceed with this method.

In conclusion, H₂S gas suicide attempts are of an extremely high mortality rate. The gas can also injure family, paramedics and caregivers. More research is needed into the potential dangers to first responders before hospitals and other agencies can make comprehensive plans about how to deal with victims. This fad spread rapidly by internet communication, and can happen anywhere in the world with chemicals readily available for purchase online.

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Authors' contributions

All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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LETTER TO THE EDITOR

Primary mucinous carcinoma of the skin with plasmacytoid cells

Dear Editor,

A 64-year-old man had noticed a tumor in his upper lip, which gradually enlarged for approximately 30 years. The biopsy specimen suggested malignancy and therefore he was introduced to us for surgery. Physical examination revealed the soft tumor on his upper lip (Fig. 1). Blood count, biochemical tests and serum tumor markers such as α -fetoprotein (AFP), carcinoembryonic antigen (CEA), neuron-specific enolase (NSE), prostate-specific antigen (PSA) and squamous cell carcinoma (SCC) were within normal limits. No lymph node swelling was palpable. Gallium and bone scintigraphy or systemic computed tomography did not show any evidence of malignant tumors. Biopsy specimen showed multiple tumor nests containing a great amount of mucinous material from dermis to subcutaneous tissue (Fig. 2a). Some parts of the tumor cells had a mildly pleomorphic and atypical nucleus (Fig. 2b) and tubular lumen formation was seen in some nests of the



Figure 1. Clinical appearance. The raised lesion with a 1.5 cm diameter was seen on the left side of the upper lip.

tumor cells (Fig. 2c). Some parts of the tumor cell nests seemed to float in the mucinous stroma (Fig. 2d). Moreover, some nests consisted of plasmacytoid cells containing round or ovoid cells with an eccentric nucleus and eosinophilic cytoplasm, which can be seen in myoepithelioma or mixed tumor of the skin (Fig. 2e). The mucous stroma was positive for periodic acid Schiff (PAS), diastase-resistant PAS (D-PAS) and Alcian blue staining at pH 2.5 but negative for AB at pH 1.0, demonstrating that the mucous material in the stroma was sialomucin. Moreover, immunohistochemically the tumor was positive for low molecular weight cytokeratins (AE1/AE3), S-100 and Her-2 but negative for CEA, GCDPF-15 and α -smooth muscle actin (α -SMA). The plasmacytoid cells showed the same staining results, indicating that these cells were simply epithelial. Epithelial membrane antigen (EMA) was only focally (<5% cells) and faintly stained in the tumor (Fig. 2f) but negatively in the plasmacytoid cells. From these findings the tumor was diagnosed as primary mucinous carcinoma of the skin (PMCS). Then, the tumor was widely excised with the orbicularis oris muscle at 1-cm margins from the raised lesion under general anesthesia. Then, additional operations with reconstruction of the upper lip and floor of nostril by bilateral lip advancement flap and Abbé lip-switch flap were successfully performed. Ten months later, a satisfactory result was observed without recurrence.

In our case, the plasmacytoid cells, corresponding with myoepithelial cells, were observed in the skin specimen. Because this type of cell can be seen in myoepithelioma, we ruled out this possibility by immunohistochemical positivity for low molecular weight cytokeratins and negativity for α -SMA. In mixed tumors of the skin, the plasmacytoid cells reportedly show the same immunoreactivity and subsequently the authors concluded these cells as the

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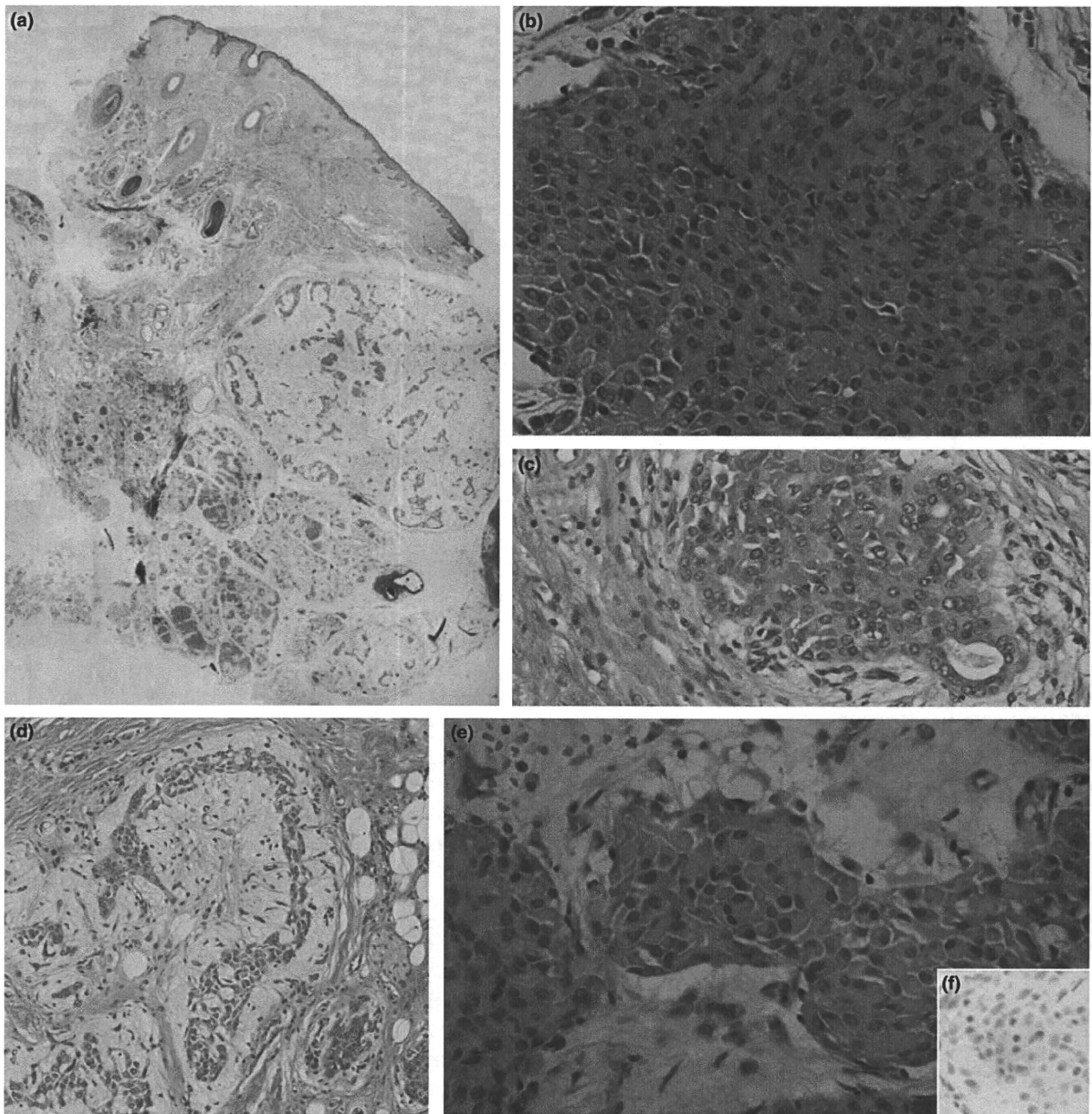


Figure 2. Histopathological findings of the skin specimen. (a) Biopsy specimen showed multiple tumor nests containing a great amount of mucinous material from dermis to subcutaneous tissue (composite image, hematoxylin–eosin [HE], original magnification $\times 10$). (b) In some parts, the tumor cells had a mildly pleomorphic and atypical nucleus (HE, original magnification $\times 40$). (c) The tubular lumen formation was seen in some nests of the tumor cells (HE, original magnification $\times 40$). (d) In some parts, the tumor cell nests seem to float in the mucinous stroma (HE, original magnification $\times 200$). (e) Plasmacytoid cells in the tumor. Some nests consist of plasmacytoid cells containing round or ovoid cells with an eccentric nucleus and eosinophilic cytoplasm (HE, original magnification $\times 40$). (f) Immunostaining of epithelial membrane antigen (EMA) of the tumor. (h) EMA was only focally ($< 5\%$ cells) and faintly stained in the tumor (original magnification $\times 200$).

simple epithelial type.¹ In addition, we discriminated this case from mixed tumor of the skin, in which plasmacytoid cells can be observed, by the presence of

sialomucin and absence of sulfated acid mucopolysaccharides as the Alcian blue staining demonstrated. Recently, in a case report of PMCS the

plasmacytoid cells were detected only by fine-needle aspiration cytology,² suggesting that the population of these cells is limited.

In our case, the skin specimen was only focally (<5% cells) and faintly stained for EMA, setting positive control for EMA using normal sebaceous glands. According to the previous study, 35 (94.6%) of tested 37 cases of PMCS showed 3+ (>50% cells positive) and the remaining two 2+ (26–50% cells positive) or 1+ (5–25% cells positive) for EMA, respectively.³ Additionally, one case report showed the focal positivity of EMA in PMCS of scalp.⁴ Therefore, although the staining intensity of EMA in our case appeared to be lowered (Fig. 2f), it does not contradict the diagnosis of PMCS. Moreover, the plasmacytoid cells were negative for EMA. In this context, the plasmacytoid SCC of the vulva has been reported to be negative for EMA⁵ although most SCC of the skin are stained positively,⁶ suggesting that the plasmacytoid feature and negativity for EMA in our case may correlate with each other.

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Letter to the Editor

Acantholytic squamous cell carcinoma of the uterine cervix

To the Editor:

Acantholytic squamous cell carcinoma is a rare variant of uterine cervical cancer. In this article we describe a case of acantholytic squamous cell carcinoma of the uterine cervix. The patient was a Japanese woman in her 50s who presented with atypical vaginal bleeding. Colposcopy indicated a thumb tip-sized soft polypoid mass that bled easily. The hysterectomy specimen contained a polypoid tumor 2 cm in size in the uterine cervix (Fig. 1). Histologically, the tumor was invasive carcinoma with marked acantholysis (Fig. 2). The tumor contained a non-keratinizing squamous cell carcinoma component. The TNM stage was IB1. On immunohistochemistry the tumor cells were focally immunoreactive for cytokeratin 5/6 (Fig. 3). The immunohistochemistry results are summarized in Table 1. Eosinophilic amorphous materials were focally stained with Congo red and had green birefringence of amyloid in polarized light, indicating slight deposition of amyloid.

In other organs including skin, vulva, and breast, the acantholytic variant of squamous cell carcinoma is described in major textbooks and is known as a rare variant of squamous cell carcinoma.^{1–6} In the uterine cervix, however, acantholytic squamous cell carcinoma is not widely known. At National Hospital Organization Kure Medical Center and Chugoku Cancer Center only one case of acantholytic variant has been reported in 203 cases of uterine cervical squamous cell carcinoma. There is only one case report of this tumor.⁷ Similarly, amyloid was demonstrated in that report.

Accurate diagnosis of acantholytic squamous cell carcinoma is difficult based only on biopsy specimens, especially when taken from the acantholytic component only. In the present case, common squamous cell carcinoma in part of the hysterectomy specimen led to the diagnosis of acantholytic squamous cell carcinoma. Differential diagnosis includes adenocarcinoma, adenosquamous carcinoma, adenoid cystic carcinoma and adenoid basal carcinoma. Adenocarcinoma and adenosquamous carcinoma have a glandular or papillary pattern.⁸ It is important to recognize acantholytic squamous cell carcinoma as one of the variants of squamous cell carcinoma of the uterine cervix for differential diagnosis. This carcinoma should be diagnosed as a variant of squamous cell carcinoma not as adenocarcinoma, because postoperative therapy could be different between squamous cell carcinoma and adenocarcinoma, especially in the late or advanced stage. Although stage is thought to be

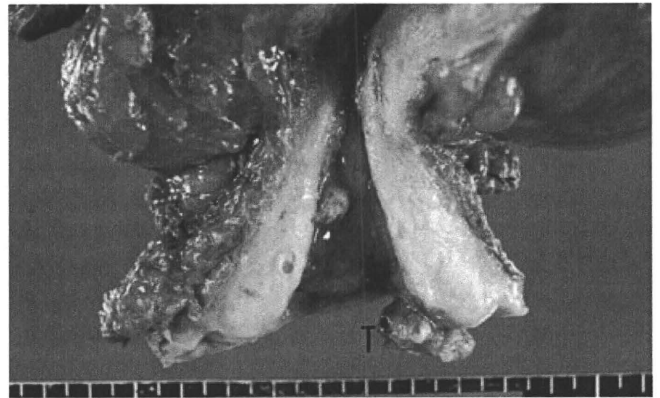


Figure 1 The tumor (T) was located at the squamocolumnar junction of the uterine cervix. The tumor was soft and its surface was rough.

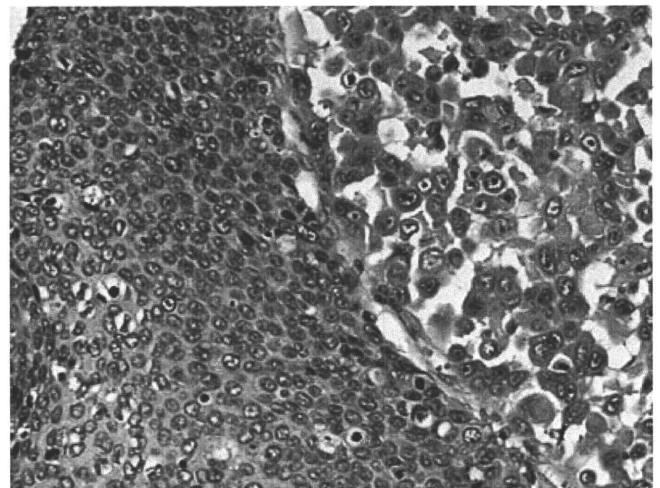


Figure 2 The tumor was adjacent to non-keratinizing squamous cell carcinoma.

Table 1 Immunohistochemistry findings

Marker	Results
Cytokeratin 5/6	+ (focal)
Cytokeratin AE1/AE3	+ (focal)
Laminin	+ (weak)
Involucrin	–
BerEP4	–
Type IV collagen	– (+, stroma)
S-100 protein	–
HHF35	–

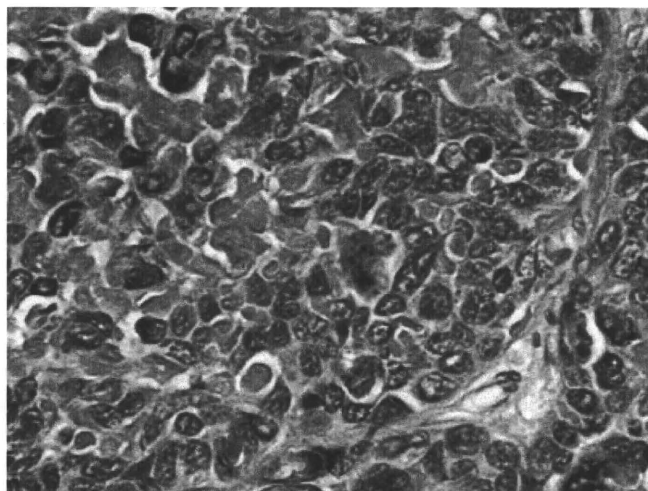


Figure 3 On immunohistochemistry the tumor cells and eosinophilic amorphous materials were focally immunoreactive for cytokeratin 5/6.

the most important prognostic factor in uterine cervical cancer, in order to examine and characterize the biological behavior, etiology, pathogenesis, and response to chemoradiotherapy and prognosis, more cases of the acantholytic squamous cell carcinoma should be studied.

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Intake of n-3 and n-6 polyunsaturated fatty acids and development of colorectal cancer**by subsite: Japan Public Health Center–based prospective study**

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Running title: Polyunsaturated fatty acids and colorectal cancer

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1 Abstract

2 To date, epidemiologic studies investigating intake of n-3 and n-6 polyunsaturated fatty acids
3 (PUFAs) and risk of colorectal cancer (CRC) are limited, and results remain inconsistent. This
4 is the first prospective study to show the association by subsite (proximal colon, distal colon,
5 rectum). To clarify the role of n-3 and n-6 PUFA intake in colon carcinogenesis, we conducted
6 a large, population-based prospective study, characterized by high fish consumption and a
7 wide range of n-3 PUFA intakes. Subjects were followed from response to a lifestyle
8 questionnaire in 1995–1999 through 2006. During 827,833 person-years of follow-up
9 (average 9.3 years), we identified 1268 new CRC cases (521 colon and 253 rectal for men;
10 350 colon and 144 rectal for women). Compared to the lowest quintile, the relative risk and
11 95% confidence interval of developing cancer among the fifth quintile of marine n-3 PUFA
12 intake were 0.60 and 0.31–1.14, respectively (P for trend = 0.04) in the colon in women and
13 0.35 and 0.14–0.88 (P for trend = 0.05) and 1.82 and 0.79–4.20 (P for trend = 0.16) in the
14 proximal and distal colon, respectively, in men. For rectal cancer, the dose response for
15 marine n-3 PUFAs was unclear; rather, we observed U-shaped associations in men and
16 women. We found no evidence that n-6 PUFA increases or the n-3/n-6 ratio decreases the risk
17 of CRC. Our results suggest that intake of marine n-3 PUFAs may be inversely related to the
18 risk of cancer in the proximal site of the large bowel.

19 Introduction

20 Conclusive evidence for the effectiveness of n-3 fatty acids (FAs) against cancer has
21 not been obtained and the mechanisms by which the FAs contribute to the prevention of
22 various cancers have not been fully established (1). The hypothesized mechanisms regarding
23 the possible role of n-3 PUFAs in the etiology of colon carcinogenesis includes an
24 anti-inflammatory effect through the suppression of arachidonic acid (AA; n-6
25 PUFA)-derived proinflammatory eicosanoids and influences on transcription factor activity,
26 gene expression, signal transduction, production of free radicals and reactive oxygen species,
27 insulin sensitivity, and so on (2). Owing to the proposed competitive role of n-3 and n-6
28 PUFAs through inflammation, the composition of these PUFAs was suggested to be a
29 biologically plausible target.

30 However, an advisory report from the American Heart Association now recognizes that
31 n-6 FAs are a beneficial part of a heart-healthy eating plan (3). According to the report, based
32 on a tracing study, the extent of conversion of linoleic acid (LA; n-6 PUFA) to AA is very low
33 (about 0.2%), and human studies showed that high plasma levels of n-6 PUFAs (mainly AA)
34 were associated with decreased serum proinflammatory markers, particularly interleukin
35 (IL)-6 and IL-1 receptor antagonists, and increased levels of anti-inflammatory markers,
36 particularly transforming growth factor- β . Mechanisms of improving insulin resistance,
37 reducing the risk of diabetes, and lowering blood pressure are also presented. Through the

38 preceding mechanisms, especially anti-inflammation or improvement in insulin sensitivity, it
39 is possible that n-6 PUFA has a rather beneficial effect on the risk of developing colorectal
40 cancer (CRC).

41 To date, epidemiologic studies investigating intake of n-3 and n-6 PUFAs and risk of
42 CRC are limited, and results remain inconsistent (4). This may be due to inaccuracy in dietary
43 assessment, an insufficient amount or variety of intake, possible potentially carcinogenic
44 substances in fish, and so on. One of the studies is our previous report on fish, marine n-3
45 PUFAs such as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) and CRC risk;
46 in this cohort, we used a concise baseline questionnaire, including 44 or 52 food items, and
47 found no association at all (5). In this present report, we used a more detailed questionnaire
48 (138 food items) at the 5-year follow-up to investigate the association between n-3 and n-6
49 PUFAs as a whole, as well as specific PUFAs, such as DHA, EPA, docosapentaenoic acid
50 (DPA), and α -linolenic acid (ALA). The substantial growth in number of observed cases (705
51 to 1268) also enabled us to conduct a more informative analysis. In this large,
52 population-based prospective study using a detailed dietary assessment tool, we attempted to
53 clarify the role of n-3 and n-6 PUFAs in colon carcinogenesis.

54

55 **Subjects and Methods**

56 **Study population**