el producto viral más abundante de la infección latente de VEB[26]. La determinación se realizó con la sonda TTGCTAGGGAGGAGACGTGT complementaria a los nucleótidos 6653-6672 del gen EBER-1 de acuerdo al protocolo descrito por Chang et al[27].

Inmunohistoquímica para la identificación de p53 y Her2/neu. La determinación de p53 se realizó por inmunohistoquímica utilizando el anticuerpo DO-7 (Dako) de acuerdo a protocolos establecidos[28]. Brevemente, se realizaron cortes de 4 µm que fueron montados en portaobjetos seguidos de desparafinación, rehidratación y digestión con Tripsina al 0,37%. A continuación, las muestras fueron incubadas con el anticuerpo DO-7 (1:10) a 4°C por 12 h seguidas de anticuerpo secundario conjugado con biotina (1:300) por 30 min a 37°C y complejo estreptavidina-peroxidasa (1:300) por 30 min a 37°C. Finalmente, las láminas fueron incubadas con diaminobenzidina (DAB) por 10 min a temperatura ambiente en presencia de H_2O_2 al 0,3% y contrateñidas con hematoxilina. La determinación de Her-2/neu se realizó con el anticuerpo policlonal pAB-1 (Dako) en condiciones similares a las descritas y considerando reacción positiva a la tinción específica en la membrana citoplasmática de células tumorales[29].

Estadística. Los resultados y las asociaciones con variables clínico-patológicas se estudiaron usando el método de chi-cuadrado (x^2) y los análisis de sobrevida usando el método de Kaplan-Meier. Estos análisis estadísticos se hicieron en el paquete computacional Stata 6.0.

Resultados

Hibridación in situ. La hibridación *in situ* se realizó en 93 casos, resultando 22 (23,6%) con tinción positiva. En los casos considerados con tinción positiva se observó la expresión de EBER-1 en forma uniforme y exclusiva en todos los núcleos de las células tumorales y no se observó expresión de EBER-1 en células epiteliales no tumorales ni linfocitos peritumorales (Figura 1).

Características clínicas y patológicas. Los resultados de la comparación de variables clínicas y anátomo-patológicas entre casos de CG cardial VEB+ y VEB- se muestran en la Tabla 1. Se observa que con relación al sexo y edad no hay diferencias significativas entre ambos grupos. Sin embargo, la edad más frecuente en que ocurrió la enfermedad, o modo, fue menor en el subtipo VEB+ (52 años vs 67 años). Con relación a variables anátomo-patológicas se observa que no hay diferencias en el tamaño tumoral como tampoco la infiltración de la pared gástrica entre los tumores VEB+ y VEB-. Entre los tumores T2-T4 (avanzados) observamos que aunque la forma Bormann III fue la más frecuente para ambos grupos, esta presentación macroscópica fue más frecuente en los tumores EBV+ (p=0,06). Con relación a la presentación histológica y basándose en la clasificación de Lauren, no se observaron diferencias entre los tipos "intestinal" y "difuso". En esta serie observamos 4 casos con abundante infiltrado linfocitario, los que fueron clasificados como linfoepitelioma gástrico y todos ellos correspondieron al grupo VEB+.

Características moleculares. La determinación de p53 se realizó en 21 casos VEB+ y en 57 casos VEB-. Se consideró tinción positiva con 10% o más

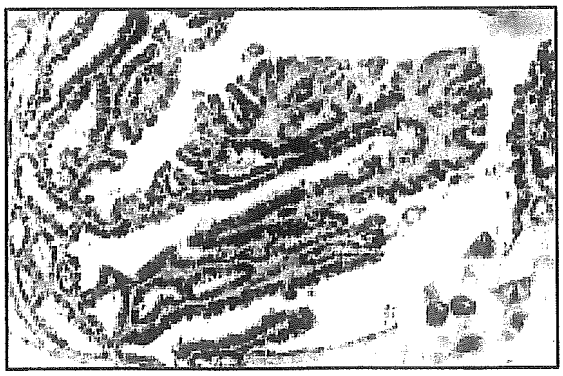

Figura 1. Hibridación *in situ* para EBER-1. Se observa expresión uniforme y exclusiva de EBER-1 en todos los núcleos de células tumorales de un cáncer gástrico cardial de tipo intestinal bien diferenciado (papilo-tubular), indicando la presencia de VEB (40X). Inserto: detalle con mayor aumento de la tinción nuclear.

Tabla 1. Correlaciones clínico-patológicas del cáncer gástrico de ubicación cardial asociado al virus de Epstein-Barr

	VEB positivo 22 casos		VEB negativo 71 casos		
	n	(%)	n	(%)	p
Sexo					
hombre	6	(27,3)	25	(35,2)	
mujer	16	(72,7)	46	(64,8)	0,49
Edad					
promedio	63,2a	(26-79a)	61,8a	(20-79a)	
Tamaño tumor					
<5 cm	4	(22,2)	26	(42,6)	
>5 cm	14	(77,8)	35	(57,4)	0,12
Pared gástrica					
T0-T1	3	(15,8)	9	(12,9)	
T2-T4	16	(84,2)	61	(87,1)	0,71
Bormann I	0	-	4	(6,6)	
Bormann II	2	(12,5)	19	(31,1)	
Bormann III	11	(68,8)	26	(42,6)	0,06[a]
Bormann IV	2	(12,5)	9	(14,8)	
Bormann V	1	(6,2)	3	(4,9)	
Linfonodos					
negativo	8	(42,1)	19	(37,3)	
positivo	11	(57,9)	32	(62,7)	0,71
Histología					
intestinal	13	(59,1)	51	(71,8)	
difuso	9	(40,9)	20	(28,2)	0,26

[a]Bormann III vs Bormann I, II, IV y V.

de células tumorales positivas a nivel nuclear (Figura 2) y los resultados se muestran en la Tabla 2. Se observa que sólo 3 (14,2%) de 21 tumores VEB+ presentaron acumulación de p53. Por el contrario, entre los tumores VEB-, 21 (36,8%) de 57 casos VEB- acumularon p53 (p=0,06). La determinación de Her2/neu se realizó en 20 casos VEB+ y 61 casos VEB-. Se consideró tinción positiva la tinción de membrana citoplasmática exclusivamente en las células tumorales (Figura 3). Los resultados se muestran en la Tabla 2. No observamos expresión de Her2/neu en ningún caso de CG cardial VEB+, y sólo en 3 (4,9%) casos VEB-.

Análisis de sobrevida. De 93 pacientes portadores de CG cardial, los datos de sobrevida fueron

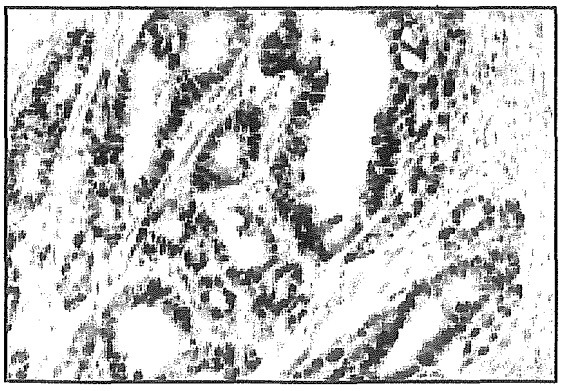

FIGURA 2. Inmunohistoquímica para proteína tumoral p53. Se observa tinción de p53 en el núcleo de células tumorales (40X).

Tabla 2. Correlaciones moleculares del cáncer gástrico cardial asociado al virus de Epstein-Barr

	VEB positivo n (%)	VEB negativo n (%)	p
p53			
negativo	18 (85,7)	36 (63,2)	
positivo	3 (14,3)	21 (36,8)	0,06
Her2/neu			
negativo	20 (100)	58 (95,1)	
positivo	0 0	3 (4,9)	0,17

FIGURA 3. Inmunohistoquímica para oncogen c-erbB-2. Se observa tinción en la membrana citoplasmática de células tumorales (40X).

obtenidos en 77 casos, 18 VEB+ y 59 VEB-. Observamos una sobrevida global de 67% y 42% para los tumores VEB+ y VEB-, respectivamente. La tendencia en el riesgo relativo de morir fue 0,57 (95% IC=0,23 a 1,41; p=0,23) para los tumores VEB- con respecto a los tumores VEB+. Para comparar la probabilidad de sobrevida en tiempos específicos entre tumores EBV+ y EBV-, realizamos un análisis de función de sobrevida (Tabla 3), que nos muestra que los tumores EBV+ tienen una mejor sobrevida inicial, pero que estas diferencias tienden a desaparecer después de los 48 meses de seguimiento.

Tabla 3. Análisis de la función de sobrevida para el virus Epstein-Barr en cáncer gástrico cardial

Tiempo	VEB-	VEB+
1 mes	100%	100%
12 meses	78,1%	94,4%
24 meses	63,7%	88,8%
36 meses	57,1%	76,1%
48 meses	52,4%	69,8%
60 meses	52,4%	62,8%
72 meses	52,4%	62,8%

DISCUSIÓN

Varias líneas de evidencias apoyan la asociación entre VEB y CG. La demostración de monoclonalidad del genoma de VEB en casos de CG indica que la infección viral precede a la expansión neoplásica[30]. Por otra parte, la presencia de anticuerpos anti-VEB elevados en pacientes con CG asociado a VEB respecto a pacientes con CG-VEB negativo o sujetos controles[30-32], y la presencia del patrón histológico denominado *lace pattern*[33], característico de esta forma tumoral, son otras evidencias que apoyan esta asociación.

Previamente hemos identificado una fuerte relación entre VEB y CG de ubicación cardial[19]. Para caracterizar esta asociación, analizamos características clínico-patológicas, moleculares y de sobrevida en una serie de 93 casos de CG de ubicación cardial de los cuales 22 (22,6%) correspondieron al tipo asociado a VEB. Al comparar las características anátomo-patológicas, llama la atención la mayor frecuencia de presentación Bormann III en los tumores VEB+. Esta observación no ha sido descrita previamente, sólo Yanai et al[34] han señalado que la presentación endoscópica más frecuente del CG asociado a VEB sería la incipiente IIc. Por otra parte, ambos tipos histológicos de Lauren estuvieron representados en proporciones similares. Esta observación es contraria a la asociación descrita con el tipo "difuso"[19] y probablemente indica que la localización y el tipo histológico serían variables independientes en el CG asociado a VEB.

La proteína p53 es una de las más importantes en carcinogénesis gástrica[35]. Esta proteína es de ubicación nuclear, es activada en respuesta a daño celular y su inactivación se asocia al

desarrollo de neoplasias[36]. La inactivación, por mutaciones puntuales o complejos con proteínas virales estabiliza a p53, con aumento de su vida media, característica que permite el uso de la inmunohistoquímica para su detección[37]. Utilizando esta metodología, la frecuencia de acumulación de p53 varía entre 23% y 61%[37]. Nuestros resultados muestran una tendencia a una baja frecuencia de acumulación de p53 en tumores asociados a VEB, los cuales son concordantes con la literatura[38,39] aunque en estos trabajos no se hace referencia a la ubicación cardial. C-erbB2 es un oncogen que codifica para receptores de factores de crecimiento y está activado por amplificación génica[29]. La amplificación de c-erbB2 es considerado el factor pronóstico molecular más importante en CG, ya que se correlaciona con invasión serosa y linfática, metástasis hepática y peritoneal y menor sobrevida a 5 años[40]. Sin embargo, no hay estudios que analicen el rol de c-erbB2 en CG asociado a VEB y nuestros resultados no demuestran una relación entre ambos, al menos en ubicación cardial.

Con relación a sobrevida, aunque no observamos diferencias estadísticamente significativas, en la sobrevida entre los tumores VEB+ y VEB-, sí observamos una tendencia a mejor pronóstico en los tumores EBV+. Esta información es concordante con la descrita por van Beek et al[41], en población caucásica y por nuestro propio grupo en una serie de 192 CG japoneses[42]. Dado que la ubicación cardial se considera de peor pronóstico[5], la observación de una tendencia a mejor pronóstico en los tumores EBV+, sería sugerente de un potencial rol específico de EBV+ en la historia natural del cáncer gástrico cardial. El análisis de la función de sobrevida, indica que la presencia de EBV sería particularmente relevante en los primeros 48 meses de seguimiento de estos pacientes.

En resumen, nuestros resultados, aunque no significativos, muestran tendencias de asociaciones clínico-moleculares y de sobrevida del CG cardial asociado a VEB. Estos resultados aportan información adicional a la caracterización del rol de VEB en CG cardial, un forma emergente de CG.

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Environmental Factors Related to Epstein-Barr Virus-Associated Gastric Cancer in Japan

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Epstein-Barr virus (EBV)-encoded small RNA can be detected in about 1-17 % of gastric carcinomas. To elucidate the lifestyles and other factors related to the EBV-associated gastric carcinoma (EBV-GC), we interviewed 43 EBV-GC cases and 162 non EBV-GC cases in Kagoshima Prefecture, Japan from 1996-2001. We mainly focused on lifestyles predominant among men because of its male predominance. Although the prevalence of smokers in EBV-GC cases was higher than among non EBV-GC cases, the difference was not significant (P=0.131). Frequent drinking of coffee and high-temperature drinks, as well as frequent intake of salty and spicy foods, were more prevalent among EBV-GC cases, but only frequent intake of salty food showed a significant difference between EBV-GC and non EBV-GC cases (P=0.026). In addition, EBV-GC cases tended to be exposed to wood dust and/or iron filings (P=0.068) and tar (P=0.097). These findings, together with a high frequency of EBV-GC among remnant cancers after partial gastrectomy, suggest an association between mechanical injuries to the stomach membrane and the high frequency of EBV-GC. The present study also showed that EBV-GC cases tended to be elder brothers/sisters (P for trend =0.029) suggesting that age at primary infection with EBV may be older in EBV-GC cases than non EBV-GC cases.

Key Words: Epstein-Barr virus, Gastric carcinoma, Lifestyles, Wood dust, Birth order

An *in situ* hybridization (ISH) of Epstein-Barr virus-encoded small RNA (EBER), which became available in the early 1990's, elucidated that EBER can be detected in about 1-17 % of gastric carcinomas (1-5). Such an Epstein-Barr virus-associated gastric carcinoma (EBV-GC) has the uniform expression of EBNA-1, BARTs, and EBERs in all carcinoma cells in addition to the episomal monoclonality of the EBV genome (6), elevated serum antibodies against the EBV-related antigens (7,8), and the unique 'lace pattern' morphology in some early-stage-EBER-positive gastric adenocarcinomas (9). These features strongly suggest an important etiological role of EBV in the development of EBV-GC.

Another epithelial malignancy, that is well known for the EBV involvement, is the undifferentiated nasopharyngeal carcinoma (NPC). The association of EBV with a high incidence of NPC in southern China is strongly suspected. In most of the countries around the world, including southern China and Japan, EBV infection takes place in the early childhood (10). Since EBV infection can be seen worldwide, and most of the people in China become infected with EBV during adolescence, involvement of other factors are suspected in NPC development. Many studies indicated that high consumption of salted fish in early childhood is involved. The prevalence of EBV infection among adults is also nearly 100% in Japan, suggesting that other factors are also involved in the development of EBV-GC.

EBV-GC is known for its male predominance (11), predisposition to gastric fundic-gland region, and relatively high frequencies in the moderately- differentiated and the poorly-differentiated solid types than in other histological types (1-5). It is also known that the proportion of EBV-GC in remnant gastric cancers, occurring in the remaining part of the stomach after partial gastrectomy, is quite high (as high as 25%) (12,13). The high frequency of EBV-GC in remnant stomach cancer suggests the possibility that mechanical injuries of the stomach membrane are involved in the development of EBV-GC. In this context, the male

predominance of EBV-GC may also suggest the association of EBV-GC with mechanical injuries of the stomach membrane related to life-styles common among men.

In the present study, we interviewed EBV-GC and non EBV-GC cases to make comparisons between them with respect to environmental factors, including life-styles and other factors.

Materials and Methods

Subjects and Interview. We tried to identify EBV-GCs in 25 hospitals in Kagoshima city and its neighboring towns concurrently during the period between 1996 and 2001. Biopsy specimens were used for the screening of EBER status, and 43 EBV-GC cases were identified. Once an EBV-GC case was identified, we sought interview from the case during his/her hospital stay. In general, we tried to interview 2-4 non EBV-GC cases per week at two major hospitals, where the majority of EBV-GC cases were identified. In small hospitals, we sought interview from a patient with gastric carcinoma in the same hospital as the corresponding EBV-GC case. Although we did not have information on the EBER expression on those gastric carcinoma cases at the interview, none of those cases turned out to be EBER positive since the frequency of EBV-GC was low. In most of the cases, interview was not refused. Interview was successful in 162 non EBV-GC cases. From the present study, we excluded remnant cancer cases and multiple cancer cases. A structured questionnaire was used at the interview, and information obtained was on number of siblings, birth order, smoking history, drinking and dietary habits, and history of occupational exposure to tar and wood dust and/or iron filings.

Histology. Gastric carcinomas were classified according to the classification scheme of the Japanese Research Society for Gastric Cancer (14). Briefly, the histological patterns were considered as follows: well-differentiated tubular adenocarcinoma (tub1), moderately differentiated tubular adenocarcinoma (tub2), solid poorly differentiated adenocarcinoma (por1), non-solid poorly differentiated adenocarcinoma (por2), signet ring cell carcinoma (sig), and mucinous carcinoma (muc). According to Lauren classification (15), intestinal-type tumors include carcinomas with types tub1, tub2, and muc; diffuse-type tumors include carcinomas with types por1, por2, and sig. The location of a tumor, defined as the predominant location of the tumor, was divided into the following three categories: cardia or upper third part, middle part and antrum or lower third part according to the guidelines of the Japanese Research Society for Gastric Cancer (16).

ISH assay to detect EBER. The ISH assay of paraffin-embedded tissue samples obtained from the biopsy specimens was conducted using a digoxigenin-labeled EBER-1 oligonucleotide probe as described before (17). A case was considered to be EBER positive based on an intensive nuclear dark purple signal under microscopy. In every ISH assay, lymph node section from a patient with infectious mononucleosis and a sense probe for EBER-1 were used as positive and negative controls, respectively. In the present study, the case with EBER-1-positive tumor cells but not in the surrounding normal epithelial cells was determined to be an EBV-GC, and we defined the case with EBER-1-negative tumor cells as a non EBV-GC.

Statistical analysis. We examined the proportion of EBV-GCs using logistic regression analysis. Gender, age, and tumor location were included in logistic models as covariates. Maximum likelihood estimates of odds ratios (OR) and corresponding 95% confidence intervals (CIs) were calculated. The P value for trend of age was calculated using age as a continuous variable in a logistic model. All the P values presented were two-sided.

Results

Clinico-pathological characteristics of the study subjects are summarized in Table I. The EBV-GC showed a male predominance (P=0.008), predisposition to middle or upper part of the stomach (P=0.002), and high frequencies in tumors of diffuse type (P=0.022). There was no difference in the age distribution between EBV-GC and non EBV-GC cases.

The results of logistic analysis adjusting for the effects of gender, age, and tumor location are summarized in Tables II-IV. Although the ever-smokers (ex-smokers and current smokers) and frequent drinking of coffee was more prevalent among EBV-GC cases, the differences were not significant (P=0.131 and 0.344, respectively). Drinking of alcohol and 10 or more cups of Japanese green tea was not related to the frequency of EBV-GCs (Table II).

Table III summarizes the results of analysis on factors possibly related to stomach membrane injury. Fre-

Table I - Clinico-pathological characteristics of the study subjects

	Total	EBV-GC (%)	non EBV-GC(%)	OR*	95%CI*
Gender (%)					
Male	144 (70)	38 (88)	106 (65)	3.5	1.3 – 9.8
Female	61 (30)	5 (12)	56 (35)	1	reference
Total	205 (100)	43 (100)	162 (100)		
Mean age (SD)	64.9 (12.1)	64.9 (13.7)	64.9 (11.7)		
Tumor location (%) #					
Cardia	37 (21)	8 (21)	29 (20)	2.7	0.9 – 8.8
Middle	76 (42)	24 (63)	52 (37)	4.6	1.7 – 12.3
Antrum	67 (37)	6 (16)	61 (43)	1	reference
Total	180 (100)	38 (100)	142 (100)		
Histology (%) #					
Intestinal	107 (60)	19 (48)	88 (63)	1	reference
Diffuse	72 (40)	21 (53)	51 (37)	2.6	1.1 – 6.0
Total	179 (100)	40 (100)	139 (100)		

** Odds ratios and corresponding 95% confidence intervals were obtained by logistic regression model using gender, age, and tumor location as covariates.*

 # Information of tumor location and histological diagnosis was not retrieved for twenty-five and twenty-six cases, respectively.

quent drinking of high-temperature drinks, as well as frequent intake of salty and spicy food, were more prevalent among EBV-GC cases but a statistically significant difference between EBV-GC and non EBV-GC was observed only in the frequent salty food intake (P=0.026). In addition, EBV-GC cases tended to be exposed to wood dust and/or iron filings, and the association was marginally significant (P=0.068). Tar exposure also seemed to be more frequent in EBV-GCs than non EBV-GCs (P=0.097).

The birth order in the siblings and the number of siblings were also examined (Table IV). EBV-GC cases tend to have early birth orders (P for trend =0.029), and tended to be the first or second born (OR=2.3; 95%CI, 1.0 - 5.0). On the other hand, there was no significant difference in the number of siblings between EBV-GC and non EBV-GC cases (P=0.366).

Discussion

This is the first study to examine the environmental factors related to the development of EBV-GC to our knowledge. The present study showed that frequent salty food intake, and maybe, wood dust and/or iron filings exposure are related to the high frequency of EBV-GCs, suggesting that mechanical injuries may be involved in the development of EBV-GC.

Although many studies including case-control, cohort, and experimental studies showed substantial evidence suggesting that the risk of gastric cancer could increase with a high salt intake (18), there was no laboratory evidence to indicate that salt *per se* is a carcinogen (19). Tatematsu et al. demonstrated that coadministration of salt promoted the carcinogenic effects of N-methyl-N-nitro-N- nitrosoguanidine (20). A high concentration of salt in the stomach damages the mucosal barrier because of the cytotoxicity of hyperosmolar stress, and leads to inflammation, erosion, and degeneration of the gastric mucosa. These damages and changes of the stomach membrane may promote the effects of food-derived carcinogens or other environmental factors including *Helicobacter pylori* infection (21). The synergistic interaction

Table II - Smoking and drinking habits, and coffee and green tea drinking in the EBV-GC and non EBV-GC cases

	EBV-GC (%)	non EBV-GC (%)	OR*	95%CI*
Smoking				
Non-smoker	6 (14)	64 (40)	1	reference
Ex-smoker[#]	14 (33)	41 (25)	2.0	0.5 – 7.8
Current smoker	23 (53)	56 (35)	2.7	0.7 – 10.1
Total	43 (100)	161 (100)	P for heterogeneity =0.315	
Alcohol drinking[$]				
Non-drinker	9 (24)	53 (36)	1	reference
Occasional drinker	11 (30)	36 (24)	0.8	0.3-2.8
Daily drinker	17 (46)	58 (39)	0.8	0.3-2.3
Total	37 (100)	147 (100)	P for heterogeneity =0.89	
Coffee				
Not often	24 (57)	107 (67)	1	reference
Often	18 (43)	52 (33)	1.5	0.6 – 3.6
Total	42 (100)	159 (100)	P =0.344	
Green tea				
<9 cups/day	31 (74)	118 (74)	1	reference
10+ cups/day	11 (26)	42 (26)	1.2	0.5 – 2.9
Total	42 (100)	160 (100)	P for trend = 0.471	

** Odds ratios and corresponding 95% confidence intervals were obtained by logistic regression model using gender, age, and tumor location as covariates.*
[#] Ex-smokers who stop smoking within 5 years were considered as current smokers.
[$] Occasional drinker: less than 5 /week; Daily drinker= 5+/ week.

between *Helicobacter pylori* infection and high-salt concentration is associated with the striking geographic variation in intestinal type of gastric tumors.

One of the drawbacks in the present study was non-quantitative information on diets. Since we asked the frequency of salty food intake based on the patients' subjective judgments, there might be some recall biases. However, this information bias might not be a differential misclassification because it was quite unlikely that the study subjects knew EBV status in their gastric tumors. Thus, we assumed that there was no difference in the degree of recall bias between EBV-GC and non-EBV-GC since all of them were patients with gastric carcinoma and interviewed during the hospital day.

We found a significant association of EBV-GC with a high frequent intake of salty food but not with other variables which are possibly related to mechanical injuries to stomach membrane. There are two possible explanations. The first is a low statistical power because of the small number of the subjects exposed to wood dust, iron filings and/or tar. The other one is that the remaining factors may not be strongly associated with the mechanical injury of stomach membrane. There are conflicting results of spicy food being the risk factor of gastric cancer, while not high temperature of hot drinks (22).

A study of Americans with Japanese ancestry living in Hawaii, conducted by Shibata et al. (23), reported 10% of 187 gastric cancer cases to be EBV-associated. The observed proportion of EBV-GC was intermediate between Japanese (7%) (2) and Americans in Los Angeles (16%) (1), suggesting that the frequency of EBV-GC cases may be affected by environmental factors. On the other hand, a study examined EBV-GC in 149 Japanese-Brazilian and 151 non-Japanese-Brazilian gastric-carcinoma cases. 5% of cases in Japanese Brazilians were EBER-positive, which was slightly lower than that of the referent Japanese, where 6% of 2,038 gastric-carcinoma cases were EBER-positive

Table III - Lifestyles possibly related to mechanical injuries to stomach membrane in EBV-GC and non EBV-GC cases

	EBV-GC (%)	non EBV-GC (%)	OR*	95%CI*
Salty food intake				
Not often	14 (33)	76 (48)	1	reference
Often	28 (67)	84 (52)	2.5	1.1 – 5.9
Total	42 (100)	160 (100)	P =0.026	
Spicy food intake				
Not often	20 (48)	93 (58)	1	reference
Often	22 (52)	67 (42)	1.6	0.7 – 3.5
Total	42 (100)	160 (100)		P =0.245
Temperature of hot drinks				
Lukewarm	6 (14)	26 (16)	1	reference
Ordinary	15 (50)	77 (48)	1.5	0.5 – 4.9
Very hot	21 (36)	57 (36)	1.7	0.5 – 5.6
Total	42 (100)	160 (100)	P for heterogeneity =0.666	
Exposure to wood dust and/or iron filings#				
No	34 (81)	148 (93)	1	reference
Yes	8 (19)	12 (8)	3.0	0.9 – 10.0
Total	42 (100)	160 (100)	P=0.068	
Exposure to tar#				
No	40 (95)	158 (99)	1	reference
Yes	2 (5)	2 (1)	7.3	0.7 – 72.9
Total	42 (100)	160 (100)	P=0.097	

** Odds ratios and corresponding 95% confidence intervals were obtained by logistic regression model using gender, age, and tumor location as covariates.*

 # Subjects who have been exposed to wood dust and/or iron filings, or tar less than 10 years were considered as no exposed.

(24). This discrepancy in the frequency of EBV-GC between Japanese living in Hawaii and Brazil may be explained by the fact that Japanese immigrants to São Paulo might have a tendency to retain their native dietary habits (24).

Another piece of evidence suggesting the involvement of environmental factors in EBV-GC is its male predominance. Most of the studies reported so far showed that the proportion of EBV-GC in men was higher than that in women. Among the highest gender ratios were 7.0 observed in Caucasians living in Los Angeles (1), and 6.2 in Russians (25). On the other hand, studies in Mexico (26) and an area near Shanghai (27) reported the gender ratio of a mere 1.2 and 1.9, respectively. Japanese Brazilians also did not show such a male predominance (24). Although the underlying mechanism of male predominance in EBV-GC is yet unknown, possible factors are the life-styles more commonly observed in males than females.

The mechanism of EBV entry into gastric epithelial cells, lacking EBV receptor, CD21, is yet to be elucidated. Takada suggested the possibility of cell-to-cell contact with virus producing cells, and the involvement of a receptor other than CD21 (28). The fact that EBV-associated cancer cannot be detected in other digestive tract organs, including colon, also indicates the importance of epithelial change(s) specific to the stomach (29). One of the differences between colon and stomach is their chance to exposure to mechanical injury. Since the stomach is the more proximally located, and is an organ where food is digested, its chance of mechanical injury is higher than in the colon. The

Table IV - Birth order and the number of siblings in EBV-GC and non EBV-GC cases

	EBV-GC (%)	non-EBV-GC (%)	OR*	95% CI*
Birth order (%)				
1st	17 (40)	48 (30)	1	reference
2nd	10 (23)	22 (14)	1.0	0.4 – 3.0
3rd	6 (14)	23 (14)	0.5	0.1 – 1.9
4-5th	5 (12)	36 (22)	0.4	0.1 – 1.3
6th+	5 (12)	32 (20)	0.4	0.1 – 1.4
Total	43 (100)	161 (100)	P for trend = 0.029	
Mean number of siblings (SD)	5.3 (2.7)	5.7 (2.3)		

** Odds ratios and corresponding 95% confidence intervals were obtained by logistic regression model using gender, age, and tumor location as covariates.*

observed association of salty food intake and wood dust and/or iron filing exposure, together with a high frequency of EBV-GCs among remnant gastric cancer (12,13), suggest that mechanical injuries to the stomach membrane may be related to the high frequency of EBV-GCs. However, this hypothesis cannot account for rare EBV-associated cancer in the esophagus. There might be multiple factors and mechanisms for EBV infection of epithelial cells. Recently, zur Hausen et al. reported that EBV can only infect neoplastic gastric cells (30). We cannot still deny the possibility that the EBV infection is just a late event in gastric carcinogenesis, and need to clarify the aetiological role of EBV infection in carcinogenesis of gastric tumors.

In the present study, we observed an association between a high EBV-GC frequency and the first or second birth suggesting that older age at primary infection with EBV might be related to the risk of EBV-GC. Gutensohn and Cole (31) also reported a similar association in Hodgkin's disease. Part of Hodgkin's disease is related to EBV infection, and the risk of Hodgkin's disease was reduced among persons who were older than their siblings. They also observed the lower risk of Hodgkin's disease among persons with 5 or more siblings than those with one or none although we observed no difference in the number of siblings between EBV-GC and non EBV-GC cases. Since primary infection with EBV in adolescence is well known to cause infectious mononucleosis, conceivably, such an explosive immune response to infection may act as a stimulator or trigger the process of carcinogenesis (31).

In conclusion, the present study has shown that frequent salty food intake and wood dust and/or iron filings exposure were related to the high frequency of EBV-GCs, suggesting that mechanical injuries may be involved in the development of EBV-GCs. It has also been suggested that older age at primary infection with EBV may be related to the EBV-GC development because the first or second birth was related to the higher frequency of EBV-GC.

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Effects of Repeated Sauna Treatment on Ventricular Arrhythmias in Patients With Chronic Heart Failure

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Background The aim of the present study was to determine whether repeated 60°C sauna treatment improves cardiac arrhythmias in chronic heart failure (CHF) patients, because ventricular arrhythmias are an important therapeutic target in CHF.

Methods and Results Thirty patients (59±3 years) with New York Heart Association functional class II or III CHF and at least 200 premature ventricular contractions (PVCs)/24 h assessed by 24-h Holter recordings were studied. They were randomized into sauna-treated (n=20) or non-treated (n=10) groups. The sauna-treated group underwent a 2-week program of a daily 60°C far infrared-ray dry sauna for 15 min, followed by 30 min bed rest with blankets, for 5 days per week. Patients in the non-treated group had bed rest in a temperature-controlled room (24°C) for 45 min. The total numbers of PVCs/24 h in the sauna-treated group decreased compared with the non-treated group [848±415 vs 3,097±1,033/24 h, $p<0.01$]. Heart rate variability (SDNN, standard deviation of normal-to-normal beat interval) increased [142±10 (n=16) vs 112±11 ms (n=8), $p<0.05$] and plasma brain natriuretic peptide concentrations decreased [229±54 vs 419±110 pg/ml, $p<0.05$] in the sauna-treated group compared with the non-treated group.

Conclusion Repeated sauna treatment improves ventricular arrhythmias in patients with CHF. (*Circ J* 2004; **68:** 1146–1151)

Key Words: Heart failure; Heart rate variability; Premature ventricular contractions; Sauna

Patients with chronic heart failure (CHF) have a high prevalence of potentially serious arrhythmias and consequently, a high incidence of sudden cardiac death[1–4] The presence of ventricular arrhythmias defines a higher-risk patient group with either ischemic or non-ischemic cardiomyopathy[5–9] Antiarrhythmic medications, such as class I drugs, have been tested in myocardial infarction survivors with depressed ventricular function and in atrial fibrillation patients with a history of congestive heart failure, and most were found not to be helpful and may even increase the occurrence of arrhythmias and cardiac mortality[10–12] Some studies have shown that amiodarone improves ventricular arrhythmias and sudden cardiac death mortality in patients with CHF, yet the improvement in total mortality remains controversial[13–15] Previous studies have demonstrated that vasodilators, such as angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, improve the prognosis and ventricular arrhythmias in patients with CHF;[16–18] therefore arrhythmia is an important target for therapy in patients with CHF.

We have used thermal therapy with a 60°C dry sauna in patients with CHF, and found that it improves hemodynamic parameters, endothelial function, and clinical symptoms in many patients[19–21] Furthermore, we have demonstrated that repeated sauna treatment improves the prognosis in hamsters with CHF[22] It is well recognized that alterations in the neural control of the heart, characterized by decreased vagal activity and relative sympathetic predominance, play a key role in the occurrence of cardiac arrhythmias in patients with CHF[23] Several studies have shown that reduced heart rate variability (HRV), determined from 24-h ambulatory electrocardiographic (ECG) recordings, is associated with a greater risk for ventricular fibrillation and poor prognosis in patients with CHF[24–27] Therefore, we prospectively investigated the effects of thermal therapy on cardiac arrhythmias and HRV in patients with CHF.

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Methods

Study Population

We studied 30 patients with CHF, aged 28–80 years (mean age: 59±3 years): 24 patients (16 men, 8 women) had idiopathic dilated cardiomyopathy and 6 (5 men, 1 woman) had ischemic cardiomyopathy. Inclusion criteria included the presence of symptomatic CHF, left ventricular ejection fraction (LVEF) <50% by echocardiography, New York Heart Association (NYHA) functional class II–III, and >200 premature ventricular contractions (PVCs) per day on 24-h Holter monitoring. Seven patients were in NYHA functional class II, and the other 23 were in class III. They were randomized into a sauna-treated group (n=20) or a non-treated group (n=10). The mean number of PVCs/24 h was 3,123±819; the mean cardiothoracic ratio (CTR) on chest radiography was 58.5±1.0% (range: 49–75%); and the mean LVEF on echocardiography was 29±2% (range:

Table 1 Baseline Clinical Characteristics of the 2 Groups

	Sauna-treated group (n=20)	*Non-treated group (n=10)*	*p value*
Age	*59±3*	*59±4*	*NS*
M/F	*14/6*	*7/3*	*NS*
DCM/ICM	*16/4*	*8/2*	*NS*
Atrial fibrillation (n)	*5*	*2*	*NS*
NYHA (I/II/III)	*0/5/15*	*0/2/8*	*NS*
Body weight (kg)	*57±3*	*53±3*	*NS*
Heart rate (beats/min)	*73±3*	*73±4*	*NS*
SBP (mmHg)	*107±4*	*108±5*	*NS*
DBP (mmHg)	*65±3*	*67±3*	*NS*
Drug therapy (%)			
Digoxin	*65*	*60*	*NS*
ACE inhibitors	*95*	*90*	*NS*
β-blockers	*55*	*40*	*NS*
Diuretics	*95*	*100*	*NS*
Nitrates	*30*	*30*	*NS*
Antiarrhythmic drugs (%)			
Mexiletine	*50*	*50*	*NS*

DCM, idiopathic dilated cardiomyopathy; ICM, ischemic cardiomyopathy; NYHA, New York Heart Association; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACE, angiotensin-converting enzyme; NS, not significant.
All values are given as the mean±SE.

10–48%). All patients were receiving maintenance doses of medications for heart failure and arrhythmias, including angiotensin-converting enzyme inhibitors, diuretics, β-blockers, digitalis and antiarrhythmic drugs (mexiletine), and they were in a stable clinical condition for 1 month before entering the study. They also did not have symptomatic arrhythmias. Their medications were unchanged for at least 1 month before or during this study. Written informed consent was obtained from all patients prior to participation, and the protocol was approved by the Ethics Committee of the Faculty of Medicine, Kagoshima University.

Sauna Treatment

Thermal therapy with a far infrared-ray 60°C dry sauna was performed as previously reported[19] Patients remained supine on a bed during the sauna for 15 min, followed by 30 min of bed rest with a blanket to keep them warm. Patients were weighed before and after the sauna treatment. Oral hydration with water was used to compensate for lost weight. Patients in the non-treated group remained supine on a bed in a temperature-controlled room (24°C) for 45 min.

Assessment of Clinical Symptoms

Clinical symptoms, such as dyspnea, fatigue, sleeplessness, edema, appetite-loss and constipation, were evaluated by a self-assessment quality of life (QOL) questionnaire[20] Each item had 4 grades: remarkably improved, improved, no change, or worsened. Patients were classified into 3 groups based on the results of the questionnaire. Patients who answered 'improved' to more than 3 items were defined as the improved group, those who answered 'worsened' for at least 1 item were defined as the worsened group, and the others were defined as the unchanged group.

Laboratory Examination

A fasting blood sample was obtained in the morning to measure plasma concentrations of neurohormonal factors, including catecholamines, atrial natriuretic peptide (ANP), and brain natriuretic peptide (BNP). Plasma catecholamine (norepinephrine, epinephrine, and dopamine) concentrations were measured with high-performance liquid chomatography, and both plasma ANP and BNP concentrations were measured by radioimmunoassay. Chest radiography (CTR) and echocardiography (LVEDD, left ventricular end diastolic dimension; LAD, left atrial dimension; LVEF) also were performed.

Ambulatory ECG Recording

Ambulatory ECG monitoring was by 2-lead 24-h Holter monitoring (DMC-4502, Nihon Koden, Tokyo, Japan). The Holter tape recordings were analyzed on a full disclosure unit that printed out each individual QRS complex for subsequent visual examination. Complete determination of PVC frequency with a description and quantification of complex forms (multiform PVCs, couplets, and ventricular tachycardia) was undertaken by manual analysis of the full disclosure data. For the purpose of this study, PVCs were defined as any beat of ventricular origin faster than the sinus rate, including the premature beats in couplets and ventricular tachycardia. Ventricular tachycardia was defined as ≥3 consecutive premature beats at a rate of ≥100 beats/min. There was an excellent correlation between the 2 observers with respect to determining the total number of PVCs ($r=0.99$), and the number of episodes of ventricular tachycardia ($r=0.99$). The technician and physician were unaware of the clinical information associated with the recording. Reproducibilities of the results of 24-h Holter monitoring performed twice were assessed in 13 patients with CHF: total beats, $r=0.99$, $p<0.0001$; PVCs, $r=0.91$, $p<0.0001$; couplets, $r=0.95$, $p<0.0001$; ventricular tachycardia, $r=0.95$, $p<0.0001$.

Analysis of HRV

Time-domain parameters of HRV were analyzed on a MARS8000 analysis system (GE Medical Systems Information Technologies, Milwaukee, WI, USA) from 2-lead 24-h Holter recordings. All tapes were manually edited for exclusion of artifacts and premature beats. A minimum of 18 h of analyzable data and a minimum of 85% successive RR intervals were required for a tape to be accepted as valid. The time interval between 2 consecutive QRS complexes was calculated as the normal-to-normal (NN) interval. Abnormal QRS complexes and RR intervals

Table 2 Frequency of Ventricular Arrhythmias and Heart Rate Variability at Baseline and After 2 Weeks in the 2 Groups

	Sauna-treated group		Non-treated group		Comparison with both groups	
	Baseline	After 2 weeks	Baseline	After 2 weeks	At baseline	After 2 weeks
PVCs/24 h (beats/24 h)	3,161±1,104	848±415**	3,048±914	3,097±1,033	NS	<0.0001
Couplets (episodes/24 h)	71±33	15±11**	69±45	87±46	NS	<0.005
VT (episodes/24 h)	20±9	4±3**	21±18	24±20	NS	<0.005
Mean RR interval (ms)	807±28	831±42	858±63	872±46	NS	NS
SDNN (ms)	113±8	142±10**	111±10	112±11	NS	<0.005

*PVCs, premature ventricular contractions; VT, ventricular tachycardia; SDNN, standard deviation of NN interval; NS, not significant. All values are given as the mean±SE; **p<0.01 vs baseline.*

Table 3 Various Parameters at Baseline and After 2 Weeks in the 2 Groups

	Sauna-treated group		Non-treated group		Comparison with both groups	
	Baseline	After 2 weeks	Baseline	After 2 weeks	At baseline	After 2 weeks
NYHA (I/II/III)	0/5/15	0/15/5**	0/2/8	0/2/8	NS	<0.005
Body weight (kg)	57±3	56±3	53±3	54±3	NS	<0.05
SBP (mmHg)	107±4	100±3	108±5	108±4	NS	NS
DBP (mmHg)	65±3	62±2	67±3	67±2	NS	NS
CTR (%)	59±1	56±2**	58±1	58±1	NS	<0.05
LVEDD (mm)	64±2	61±2*	64±3	64±3	NS	NS
LAD (mm)	46±2	44±2	47±2	46±2	NS	NS
LVEF (%)	29±2	33±2*	29±3	31±3	NS	NS
NE (pg/ml)	431±56	415±76	414±42	455±84	NS	NS
EP (pg/ml)	25.3±4.1	25.0±3.4	24.9±4.3	28.3±6.0	NS	NS
DOPA (pg/ml)	13.7±3.1	13.7±3.0	14.2±4.2	14.2±3.2	NS	NS
ANP (pg/ml)	121±23	81±19**	126±32	130±37	NS	NS
BNP (pg/ml)	425±102	229±54**	415±98	419±110	NS	<0.01

*NYHA, New York Heart Association; SBP, systolic blood pressure; DBP, diastolic blood pressure; CTR, cardiothoracic ratio; LVEDD, left ventricular end diastolic dimension; LAD, left atrial dimension; LVEF, left ventricular ejection fraction; NE, norepinephrine; EP, epinephrine; DOPA, dopamine; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; NS, not significant. All values are given as the mean±SE; *p<0.05 vs baseline, **p<0.01 vs baseline.*

were replaced by a linear interpolation algorithm. The standard deviation (SD) of all normal beat intervals and the mean length of the NN intervals (SDNN) were used for time-domain measures from the entire recording period. We analyzed 24 patients; 6 patients with atrial fibrillation were excluded.

Study Protocol

Sauna treatment was performed daily for 5 days each week, for a total of 2 weeks. All examinations were performed before the first treatment and on the day after the last treatment.

Statistical Analysis

All data are expressed as the mean±SEM. Differences in baseline characteristics were evaluated by the chi-square test and unpaired t-test. Within-group changes between baseline and after 2 weeks were evaluated by paired t-test or Wilcoxon signed rank test for variables that were not normally distributed. Between-group comparisons were evaluated by Mann-Whitney's U test using differences between baseline and after 2 weeks. A value of $p<0.05$ was considered statistically significant.

Results

Baseline Clinical Characteristics and Assessment of Clinical Symptoms

Baseline clinical characteristics are summarized in Table 1. There were no differences in age, gender, NYHA functional class, mean heart rate, blood pressure or use of drugs, such as digoxin, angiotensin-converting enzyme inhibitor, β-blockers, diuretics, nitrates, and antiarrhythmic drugs, at baseline between the 2 groups. All patients enrolled completed the study. In the sauna-treated group, no patient experienced dyspnea, angina pectoris or palpitations. Clinical symptoms related to dyspnea, fatigue, edema, appetite-loss, constipation and insomnia were improved in 17 of 20 patients and unchanged in 3 patients after the 2-week sauna treatment. However, no patients had worsening of clinical symptoms. In the non-treated group, clinical symptoms did not change after 2 weeks.

Cardiac Arrhythmias

At baseline, the total number of PVCs, couplets and episodes of ventricular tachycardia per day were similar between the 2 groups (Table 2). In the sauna-treated group, the total number of PVCs decreased in all patients 2 weeks after treatment. The total number of PVCs in the sauna-treated group was significantly decreased compared with the non-treated group after 2 weeks ($p<0.01$, Table 2). The total number of couplets and episodes of ventricular tachycardia per day also decreased significantly in the sauna-treated group compared with the non-treated group (Table 2). The prevalence of couplets and ventricular tachycardia in the sauna-treated group compared with the non-treated group was 45% vs 90%, $p<0.05$, and 20% vs 80%, $p<0.01$, respectively. The total number of PACs did not significantly change between the 2 groups after 2 weeks (170±102 vs 617±375, $p=0.07$).

HRV

There was no difference in SDNN at the baseline between the 2 groups, but after 2 weeks, SDNN was sig-

nificantly greater in the sauna-treated group compared with the non-treated group (Table 2).

Neuro-Hormonal Factors

At baseline, there were no differences in the plasma concentrations of ANP, BNP, or catecholamine between the 2 groups. After 2 weeks, there were no differences in the plasma concentrations of ANP or catecholamine between the 2 groups, but the plasma concentration of BNP in the sauna-treated group was significantly lower than in the non-treated group (229±54 pg/ml vs 419±110 pg/ml, $p<0.05$; Table 3).

NYHA Functional Class, Chest Radiography, Echocardiography and Laboratory Parameters

At baseline, there were no differences in NYHA functional class, CTR or LVEDD between the 2 groups, but after 2 weeks, there was a significant difference in NYHA functional class, body weight, and CTR in the sauna-treated group; LVEDD did not change between the 2 groups. Laboratory parameters, including liver function tests (aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, γ-glutamyl transpeptidase etc), creatinine, electrolytes (Na, Cl, K) and hematocrit, did not change after 2 weeks in either group (data not shown).

Discussion

In the present study, we found that repeated 60°C sauna treatment improved ventricular arrhythmias. Furthermore, we observed that thermal therapy increased HRV and reduced the plasma concentration of BNP in patients with CHF.

The incidence of ventricular arrhythmias is extremely high in patients with CHF: approximately 80% or more of CHF patients have frequent ventricular premature beats and approximately 50% of them have runs of nonsustained ventricular tachycardia[3,28–30] Sudden death because of ventricular arrhythmias accounts for approximately half of all deaths in patients with CHF[4,31–33] Several studies have shown an association between ventricular arrhythmias and mortality in patients with CHF[5–9,34–36] but unfortunately, current antiarrhythmic medications, such as class I drugs, have only limited efficacy in these patients and may even be associated with worsening ectopic activity and hemodynamic deterioration[10–12] In large randomized trials with amiodarone, a potent antiarrhythmic drug with additional sympatholytic and minor negative inotropic effects, the Group for the Study of Survival in Heart Failure in Argentina (GESICA) demonstrated that low doses reduced ventricular arrhythmias and mortality in patients with CHF;[13] however, the Survival Trial of Antiarrhythmic Therapy in Congestive Heart Failure had conflicting results concerning mortality[14] Previous studies have demonstrated that β-blockers, which also have antiarrhythmic effects, reduce mortality and the risk of sudden cardiac death, as well as ventricular arrhythmias, in patients with CHF[37] and other studies have shown that ventricular arrhythmias in patients with CHF are improved by treatment with non-antiarrhythmic drugs, such as angiotensin-converting enzyme inhibitors[16–18] and spironolactone[38] Our present results demonstrated that thermal therapy reduced the total number of PVCs, couplets, and episodes of ventricular tachycardia in patients with CHF and we have already shown that thermal therapy reduced mortality in hamsters with CHF[22] We suggest that improvement of ventricular arrhythmias may be one of the mechanisms by which repeated thermal therapy improves the prognosis in patients with CHF.

Although the mechanisms of ventricular arrhythmias occurring in patients with CHF are still unclear, experimental evidence suggests that the development of delayed and early afterdepolarization-induced triggered activity and automaticity, in addition to conditions favoring reentry, are related to arrhythmias in the setting of heart failure. Modulating factors, such as sympathetic activation, electrolyte disturbances and chronic left ventricular stretch, are also present in the setting of heart failure[39,40] It is well-established that the sympathetic nervous system is activated in patients with CHF[41–43] and analysis of HRV provides important information about sympathetic nervous activity in these patients[25,44] Data from the recent United Kingdom-Heart failure Evaluation and Assessment of Risk Trial (UK-HEART) suggest that reduced HRV, analyzed by a traditional time-domain method (including SDNN), is related to the risk of ventricular arrhythmias and sudden death in patients with CHF[24] and we suggest that one of the mechanisms by which repeated sauna treatment significantly improves ventricular arrhythmias is by increasing HRV, although we have not clarified the underlying mechanisms of that effect of thermal therapy. On the other hand, the self-assessment QOL questionnaire revealed 17 of 20 patients who answered 'improved' to more than 3 of 6 clinical symptoms that comprised dyspnea, fatigue, sleeplessness, edema, appetite-loss and constipation, and furthermore, none of the patient answered 'worsened' for any symptom. Therefore, the improvement may be related to better mood as a result of repeated sauna treatment. Further study is needed.

The chronic stretch of cardiac myocytes contributes to shortening of the action potential duration and mild decreases in the action potential amplitude and resting membrane potential[45] These changes may be arrhythmogenic by increasing reentry and abnormal automaticity[46] In patients with CHF, the ventricular wall is chronically stretched because of increases in ventricular volume and/or pressure overload. It is well-established that BNP is secreted predominantly by the ventricle in response to ventricular wall stretch[47] On the basis of our findings, including previous data[20] which showed significantly decreased plasma concentrations of BNP after 2 weeks of sauna treatment, we speculate that another mechanism responsible for decreased ventricular arrhythmias may be reduction of ventricular wall stretch.

Electrolyte disturbances, such as hypokalemia and hypomagnesemia, are prevalent in patients treated with diuretics and are implicated as a cause of ventricular arrhythmias associated with CHF. However, we did not observe significant changes in the electrolyte concentrations after 2 weeks (data not shown).

We have treated many CHF patients with sauna therapy and so far none of the in-hospital patients has shown any deterioration in their condition. However, thermal therapy does not appear to be indicated for CHF patients with aortic stenosis or obstructive hypertrophic cardiomyopathy because the pressure gradient is increased. In the present study, only CHF patients with NYHA functional class II or III underwent sauna treatment. It is well-known that the more severe the CHF, the more prevalent are ventricular arrhythmias. We evaluated the effects of sauna therapy on

ventricular arrhythmias at 2 weeks, but further studies of the long-term effects and benefit in CHF patients with NYHA functional class IV are needed.

In conclusion, repeated 60°C sauna treatment decreased ventricular arrhythmias in CHF patients with NYHA functional class II or III.

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Epstein-Barr virus-associated gastric carcinoma in Papua New Guinea

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Abstract. Using *in situ* hybridization assay, we examined Epstein-Barr virus (EBV) encoded RNA (EBER) expression in 66 cases of oral cancer, 40 esophageal cancer cases, 150 stomach cancer cases, and 46 colorectal cancer cases diagnosed in the Pathology Department of Port Moresby General Hospital, University of Papua New Guinea during the period between 1986-2002. There were no malignancies with positive EBER expression except for the following two male stomach cancer cases: a male case with a gastric carcinoma in pylorus whose age was unknown; and a male case aged 55 years without information on location of tumor. Both cases were histologically classified as non-solid poorly differentiated adenocarcinoma of the Japanese histological classification. The frequency of EBV-associated gastric carcinomas was 1.3% (2/150), and was the lowest ever reported in the world. We examined genotypes of two EBV strains detected from gastric carcinomas. Four different regions of EBV genome were examined by PCR-RFLP, coupled with Southern blot hybridization. The EBV genotype of the first case were type A, wild-type F at BamHI-F region, type D of BamHI-I region and the kept type of the XhoI cleavage site in LMP1. The second case had EBV whose genotypes were type A, wild-type F at BamHI-F region, and the kept type of the XhoI cleavage site in LMP1. The BamHI-I region of this case could not be analyzed.

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Key words: Epstein-Barr virus, gastric carcinoma, Papua New Guinea

Introduction

In 1992, Shibata and Weiss (1) reported the presence of Epstein-Barr virus (EBV) genome in 16% of gastric adenocarcinomas in a small North American series, using *in situ* hybridization technique to detect EBV-encoded small RNA (EBER) genome in gastric tissue. A large-scale study in Japan, published in 1993, also showed the presence of EBER in 7% of gastric carcinomas (2). Subsequent studies revealed that the proportion of EBV-associated gastric carcinoma (EBV-GCs) was different from country to country, and ranged from 2 to 17% (3). In the present study, we examined the prevalence of EBV-GCs in Papua New Guinea (PNG).

Materials and methods

Subjects. The present study examined cancer cases of digestive organs diagnosed in Pathology Department of Port Moresby General Hospital, the major teaching center of School of Medicine and Health Sciences, University of Papua New Guinea. Paraffin-embedded formalin-fixed tissues of the following cancers were examined: 66 cases of cancer of the oral cavity diagnosed in 2001; 40 cases of esophageal cancer for the period 1994-2002; 150 stomach adenocarcinoma cases for the period 1986-2002, and 46 colorectal cancer cases for the period 1989-2002.

Histological classification. Histological classifications of oral and esophageal cancers were made following the guidelines of Japan Society for Head and Neck Cancer (4), and Japanese Society for Esophageal Diseases, respectively (5). The gastric carcinomas were classified as the intestinal- and diffuse-type of Lauren classification (6), and subclassified according to the Japanese Classification of Gastric Carcinoma of Japanese Research Society for Gastric Cancer (7). Briefly, histological patterns were classified as follows: well differentiated tubular adenocarcinoma (tub1), moderately differentiated tubular adenocarcinoma (tub2), solid poorly differentiated adenocarcinoma (por1), non-solid poorly differentiated adenocarcinoma (por2), signet ring cell carcinoma (sig), and