



• GASTRIC CANCER •

Epstein-Barr virus-associated gastric carcinoma in Kazakhstan

Gabit Alipov, Toshiyuki Nakayama, Masahiro Nakashima, Chun-Yang Wen, Daisuke Niino, Hisayoshi Kondo, Yuri Pruglo, Ichiro Sekine

Gabit Alipov, Masahiro Nakashima, Ichiro Sekine, Tissue and Histopathology Section, Division of Scientific Data Registry, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Toshiyuki Nakayama, Chun-Yang Wen, Daisuke Niino, Ichiro Sekine, Department of Molecular Pathology, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Hisayoshi Kondo, Biostatistics Section, Division of Scientific Data Registry, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Yuri Pruglo, Department of Pathology, Semipalatinsk Medical Academy, Semipalatinsk, Kazakhstan

Correspondence to: Toshiyuki Nakayama, M.D., PhD, Department of Molecular Pathology, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan. toshi-n@net.nagasaki-u.ac.jp

Telephone: +81-95-8497107 **Fax:** +81-95-8497108

Received: 2004-05-10 **Accepted:** 2004-07-09

Abstract

AIM: To investigate the incidence of Epstein-Barr virus-associated gastric cancer (EBV-GC) in Kazakhstan and to compare it with that in Russia, Western and Asian countries in order to evaluate the significance of epidemiopathologic and ethnic factors.

METHODS: *In situ* hybridization (ISH) of EBV-encoded small RNA-1 (EBER-1) was used to identify the presence of EBER-1 signal in 139 formalin-fixed and paraffin-embedded GC tissues from Kazakhstan.

RESULTS: EBER-1 expression was observed in the nuclei of 10% of the cases of GC (14/139), but not in the surrounding normal mucosa. The incidence of the diffuse type of EBV-GC was significantly higher in Kazakhstan (14%, 13/91) than that of the intestinal type (2%, 1/48). Furthermore, the incidence was significantly higher in males (14%, 12/89) than in females (3.7%, 2/53) from all countries. The overall incidence of EBV-GC increased from 6.7% in Asian countries to 8.7% in Russia, 10.1% in Kazakhstan and 16% in Western countries.

CONCLUSION: Geographical differences in the incidence of EBV-GC may reflect the epidemiologic factors and/or dietary habits independent of histological type and sex.

© 2005 The WJG Press and Elsevier Inc. All rights reserved.

Key words: Gastric carcinomas; Epstein-Barr virus; EBV-encoded small RNA-1

Alipov G, Nakayama T, Nakashima M, Wen CY, Niino D, Kondo H, Pruglo Y, Sekine I. Epstein-Barr virus-associated gastric carcinoma in Kazakhstan. *World J Gastroenterol* 2005; 11 (1): 27-30

<http://www.wjgnet.com/1007-9327/11/27.asp>

INTRODUCTION

It is well established that EBV is associated with several malignant diseases. The Epstein-Barr virus (EBV), a widely distributed gamma herpes virus, is directly implicated in the pathogenesis of a variety of lymphoproliferative and neoplastic disorders, including Burkitt's lymphoma, B-cell lymphomas in immunosuppressed patients and epithelial malignancies, e.g., nasopharyngeal carcinoma^[1,2]. EBV-associated carcinomas have also been described in salivary, parotid glands^[3,4] and gastric cancers^[5] based on the expression of the virus in these tissues.

Although gastric cancer is one of the most common malignant diseases in Kazakhstan, the proportion associated with EBV is significantly higher in the United States (16%)^[5] and Russia (8.6%)^[6], in comparison with Japan (6.7%)^[7]. Presence of EBV is significantly more frequent in males and in cardiac tumors of the stomach. The incidence is highest in undifferentiated lymphoepithelioma-like carcinomas (80-90%), followed by moderately differentiated tubular adenocarcinomas and poorly differentiated solid types^[7-9]. *In situ* hybridization has revealed positive lesions in almost all carcinoma cells coupled with the absence of signal in the surrounding normal stromal cells and gastric mucosa^[5-9].

The purpose of this study was to determine the incidence of EBV-infected GC in Kazakhstan. Its geographical location between West and East makes it amenable for comparison between the two regions. Gastric cancer is extremely malignant with a poor prognosis and is the second leading cause of cancer-related deaths in Kazakhstan with an annual mortality rate of 10.6% per 100 000 persons^[10].

MATERIALS AND METHODS

Patients and samples

In this study, tissues from 139 patients treated at regional hospitals in the East Kazakhstan region of Semipalatinsk between 1996 and 1998 were analyzed. Formalin-fixed and paraffin-embedded tissues from 100 surgical and 39 biopsy cases were used for the investigation, including samples from 86 males and 53 females. Information pertaining to age, sex, the primary site of cancer, histological type and race were collected.

Histological type of samples

Histologic specimens were fixed in 40 g/L formaldehyde formalin and routinely processed for paraffin-embedment. Histological sections (4 µm) were stained with hematoxylin and eosin and subjected to *in situ* hybridization (ISH) for EBV-encoded small RNA-1.

All the cases were classified histologically as either intestinal or diffuse type GC according to the Lauren classification^[11]. We also employed the Japanese Research Society of Gastric Cancer classification scheme^[12]. The intestinal type included well and moderately differentiated tubular adenocarcinomas, tub1 and tub2 respectively, whereas the diffuse type consisted of solid and non-solid poorly differentiated adenocarcinomas, i.e., por1 and por2, as well as signet-ring cell (sig) and mucinous (muc) carcinomas (Table 1). Poorly differentiated lymphoepithelioma-like carcinomas were excluded from this study. The surgical cases

Table 1 Incidence of EBER-1 expression in gastric carcinoma by histological type

Histological type	Male Positive/tested (%)	Female Positive/tested (%)	Total Positive/tested (%)
Intestinal	1/30 (3.3)	0/18 (0)	1/48 (2.1)
Tub1	0/7 (0)	0/11 (0)	0/18 (0)
Tub2	1/23 (4.3)	0/7 (0)	1/30 (3.3)
Diffuse	11/56 (19.6)	2/35 (5.7)	13/91 (14.2)
Por1	7/16 (43.7)	2/13 (15.3)	9/29 (31.0)
Por2	4/22 (18.1)	0/6 (0)	4/28 (14.2)
Sig	0/15 (0)	0/12 (0)	0/27 (0)
Muc	0/3 (0)	0/4 (0)	0/7 (0)

Table 2 Clinicopathological characteristics of EBV incidence in gastric cancer patients

	Intestinal type Positive/tested (%)	Diffuse type Positive/tested (%)	Total Positive/tested (%)
Age (yr)			
20-39	1/2 (50.0)	2/15 (13.3)	3/17 (17.6)
40-59	1/20 (5.0)	5/45 (11.1)	6/65 (9.2)
≥60	0/26 (0)	5/31 (16.1)	5/57 (8.7)
Race			
Asian	0/16 (0)	5/40 (12.5)	5/56 (8.9)
Caucasian	1/32 (3.1)	8/51 (15.6)	9/83 (10.8)
Sex			
Male	1/30 (3.3)	11/56 (19.6)	12/86 (13.9)
Female	0/18 (0)	2/35 (5.7)	2/53 (3.7)
Location			
Upper	1/15 (6.6)	5/33 (15.1)	6/48 (12.5)
Lower	0/19 (0)	8/40 (20.0)	8/59 (13.5)
Unknown	0/14 (0)	0/18 (0)	0/32 (0)

were all classified as invasive carcinomas of stomach. Tumor locations were classified as upper (fundic gland area), lower (antral gland area) and unknown regions of stomach (Table 2).

In situ hybridization

EBV was identified by the expression of EBV-encoded small RNA-1, the most abundant viral product in latently infected cells^[13-15]. EBER-1 expression was detected using a complementary digoxigenin-labeled 30-base oligomer as previously described^[16]. Briefly, 4 μm paraffin sections were cut from the main tumor, deparaffinized, rehydrated, predigested with pronase, prehybridized, and hybridized overnight at 37 °C with 0.5 mg/L of digoxigenin-labeled probes. After washing, the hybridization signal was detected using an anti-digoxigenin antibody-alkaline phosphatase conjugate based on the manufacturer's instructions (Boehringer, Mannheim, Germany).

Statistical analysis

Logistic analysis was performed to compare the proportion of EBV-positive GC cases with respect to tumor location and histological type. Gender, ethnic group and age were included as independent variables. Maximum likelihood estimates of odds ratios (OR) and corresponding 95% confidence intervals (CI) were obtained by logistic analysis using SAS statistical package for analysis of epidemiological data^[17,18].

RESULTS

A positive ISH signal was observed in 14 of 139 (10.1%) cases who were classified as EBV-associated GC (Figures 1,2). The ISH signals were specifically localized at the nuclei of tumor cells, but were absent in the surrounding normal gastric mucosa and tumor infiltrating lymphocytes (Figures 1,2).

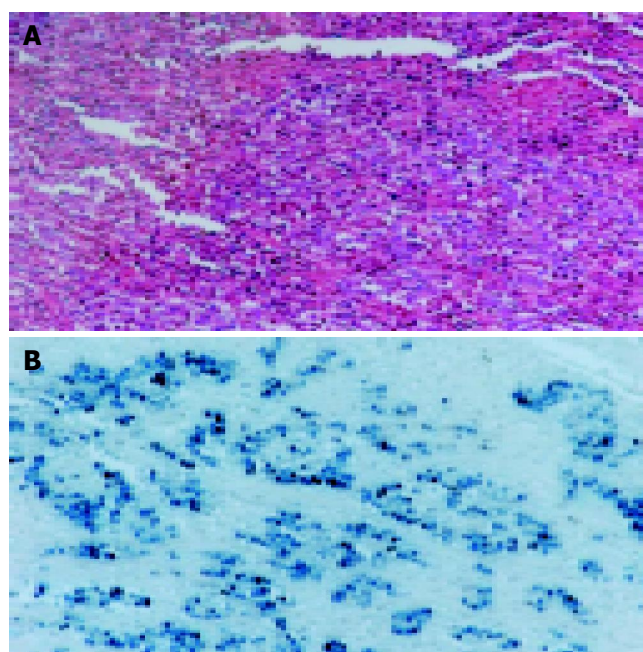


Figure 1 Strong expression of EBER-1 in nuclei of gastric adenocarcinomas coupled with the absence of signal in the stromal lymphocytes. A: H.E; B: *in situ* hybridization ×100.

Histological type

On the basis of Lauren's histological classification, 1 of 48 (2.1%) of the intestinal-type and 13 of 91 (14.2%) diffuse-type GCs were EBER-1 positive (Table 1). The proportion of EBV positive cases in the diffuse-type GC was significantly higher than that in the intestinal type (OR 8.07, 95% CI 1.43-152.8). According to

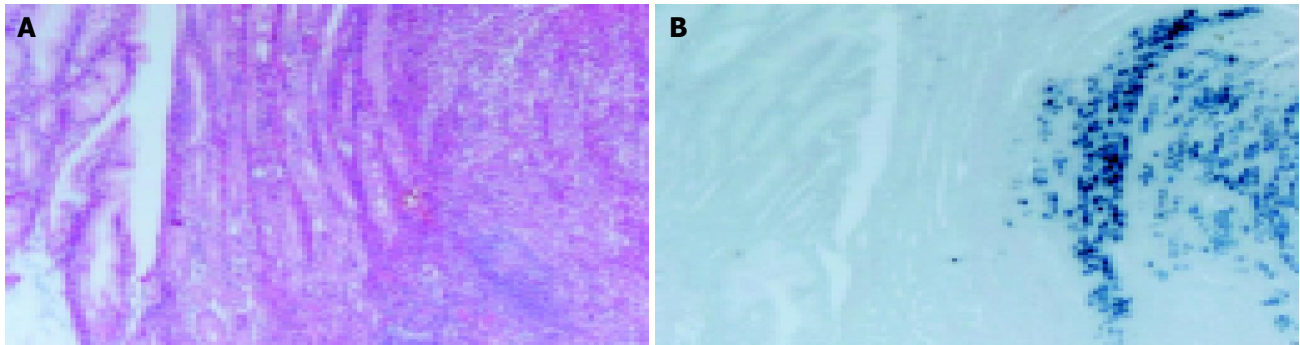


Figure 2 Strong expression of EBER-1 in nuclei of gastric adenocarcinomas coupled with absence of signals in surrounding normal gastric mucosa. A: H.E; B: *in situ* hybridization $\times 40$.

the Japanese Research Society for Gastric Cancer classification, the number of EBER-1-positive cases was higher in the solid than in the other types (OR 52.3, 95% CI 4.23-648.1). EBER-1-positive signals were absent in 18 cases of tub1, 27 cases of signet-ring cells and 7 cases of mucinous-type GCs (Table 1).

Location

Six of 48 (12.5%) cardiac tumors and 8 of 59 (13.5%) antral tumors were EBER-1 positive (Table 2). Logistic analysis did not reveal any significant differences between tumor locations and EBV infection in GCs (OR 0.94 95% CI 0.27-3.14).

Gender and age

EBER-1-positive cases accounted for 13.9% (12/86) cases in males and 3.7% (2/53) cases in females. The distribution of EBV was highest in the diffuse type (11/56, 19.6%) in comparison with the intestinal type (1/30, 3.3%) in males. Furthermore, the number of EBV-positive cases in males was significantly higher than that in females (OR 5.69, 95% CI 1.31-41.2).

The incidence of EBV-associated GC was slightly higher in young patients, aged 20-39 (3/17, 17.6%) than in those aged 40-59 (6/65, 9.2%), 60 and more than that (5/57; 8.7%). Logistic analysis did not reveal any significant differences between age correlation and EBV infection in GC. However, patients aged 60 years and older exhibited a slightly lower incidence of EBV infection as compared with the younger patients (OR 0.97, 95% CI 0.93-1.02) (Table 2).

Ethnic group

A total of 56 (41.7%) of 139 GC cases occurred in Asians, whereas 83 (58.2%) of 139 GC cases occurred in Caucasians. Although the EBV infection rate was slightly higher in Caucasians (9/83, 10.8%) than in Asians (5/56 and 8.9%), this difference was not statistically significant (OR 1.76, 95% CI 0.52-6.68).

DISCUSSION

The relationship between EBV and gastric carcinoma has been reported, i.e., EBV is detected internationally in approximately 2-18 % of gastric carcinomas^[19]. EBV infection occurred in 13 (10.1%) of 139 cases of gastric carcinomas obtained from Kazakhstan. The distribution of EBER-1 expression with respect to sex, histological type and cellular localization approximates that reported previously for gastric cancers.

The Republic of Kazakhstan is located in Central Asia and its population is approximately less than 50% of Caucasians. In this study 58.2% of the cases of GC occurred in Caucasians compared with 41.7% in the non-Caucasian population. However, there was no significant ethnic-related difference in the incidence of EBV-associated GC.

Approximately 5-7% of gastric cancers are EBV-associated in Japan, a country with a high incidence of gastric cancer. The incidence of EBV-associated gastric cancer in the United States is much higher (16%) than in Japan, whereas the incidence is intermediate in Russia (8.7%)^[6]. Japanese living in the United States also exhibit a higher incidence of EBV-associated gastric cancer than cohorts in Japan^[20], and Chinese living in Taiwan also exhibit a higher incidence of EBV-associated gastric cancer^[21] than Chinese living in Suzhou, China^[22], suggesting that the etiology of EBV-associated gastric carcinoma is influenced by environmental and cultural factors. Differences in the subtype frequency have been reported from different geographic areas^[23] and for the status of patients' immune system^[24]. Most cases of nasopharyngeal or gastric carcinoma in immunocompetent patients in Asia contain type A EBV^[21,24,25].

In the present study, the rate of EBER-1 expression in GC was intermediate between those found from patients in Western countries, Russia and Eastern countries. However, there was no positive correlation between EBER-1 expression and ethnicity. Most studies have reported that the percentage of EBV-associated gastric carcinomas is higher in males than in females. The greater prevalence of gastric cancer and EBV-associated gastric carcinoma in males suggests that risk factors or precursor lesions are related to the etiology of EBV-associated gastric carcinoma^[5,7,20]. However, we also found the EBV-positive cases in males were significantly higher than in female cases of GC. Furthermore, several reports have suggested that the incidence of EBV-positive tumors is greater in the upper stomach in comparison with the lower stomach^[26,27]. However, we did not find significant difference between location of tumor and EBV infection.

A number of studies have described the clinicopathological and biological characteristics of GCs unique to young patients in comparison with older subjects^[28-31]. Approximately 1.1-1.6% of all patients diagnosed with gastric adenocarcinoma are less than 30 years of age^[28,29]. It has long been suspected that young patients with gastric cancer have different biological features, with a more aggressive disease course and a poorer prognosis than older patients^[32].

In the present study, gastric cancer occurred in 17 (12.2%) of 139 patients less than 30 years of age. Interestingly, 15 (88.2%) of these cases were diagnosed as the diffuse type of gastric cancer. Previous studies noted that the age of the patients correlated with the rate of EBV-positive tumors; those aged 60 years and older exhibited a higher frequency of EBV-infected carcinomas^[33]. However, we did not detect a significant correlation between patient age and the incidence of EBV-associated gastric cancer.

Our study is the first to describe the incidence of EBV-associated gastric cancer incidence in Kazakhstan. The data

suggest that the geographical difference in the incidence of EBV-associated gastric cancer may reflect the epidemiological factors and dietary habits, but appears to be independent with respect to the histological type of tumor, patient gender and ethnic factors. Additional studies are necessary to clarify the epidemiology and etiology of EBV-associated gastric cancer in Central Asia.

REFERENCES

- 1 **de-The G**, Ambrosioni JC, Ho HC, Kwan HC. Lymphoblastoid transformation and presence of herpes-type viral particles in a Chinese nasopharyngeal tumour cultured in vitro. *Nature* 1969; **221**: 770-771
- 2 **zur Hausen H**, Schulte-Holthausen H, Klein G, Henle W, Henle G, Clifford P, Santesson L. EBV DNA in biopsies of Burkitt tumours and anaplastic carcinomas of the nasopharynx. *Nature* 1970; **228**: 1056-1058
- 3 **Tsai CC**, Chen CL, Hsu HC. Expression of Epstein-Barr virus in carcinomas of major salivary glands: a strong association with lymphoepithelioma-like carcinoma. *Hum Pathol* 1996; **27**: 258-262
- 4 **Gallo O**, Santucci M, Calzolari A, Storch OF. Epstein-Barr virus (EBV) infection and undifferentiated carcinoma of the parotid gland in Caucasian patients. *Acta Otolaryngol* 1994; **114**: 572-575
- 5 **Shibata D**, Weiss LM. Epstein-Barr-virus-associated gastric adenocarcinoma. *Am J Pathol* 1992; **140**: 769-774
- 6 **Galetsky SA**, Tsvetnov VV, Land CE, Afanasieva TA, Petrovichev NN, Gurtsevitch VE, Tokunaga M. Epstein-Barr-virus-associated gastric cancer in Russia. *Int J Cancer* 1997; **73**: 786-789
- 7 **Tokunaga M**, Land CE, Uemura Y, Tokudome T, Tanaka S, Sato E. Epstein-Barr virus in gastric carcinoma. *Am J Pathol* 1993; **143**: 1250-1254
- 8 **Oda K**, Tamaru J, Takenouchi T, Mikata A, Nunomura M, Saitoh N, Sarashina H, Nakajima N. Association of Epstein-Barr virus with gastric carcinoma with lymphoid stroma. *Am J Pathol* 1993; **143**: 1063-1071
- 9 **Imai S**, Koizumi S, Sugiura M, Tokunaga M, Uemura Y, Yamamoto N, Tanaka S, Sato E, Osato T. Gastric carcinoma: monoclonal epithelial malignant cells expressing Epstein-Barr virus latent infection protein. *Proc Natl Acad Sci U S A* 1994; **91**: 9131-9135
- 10 Cancer morbidity and mortality Kazakhstan population in 2002. Department of Cancer Registry in Health Ministry, *Astana*, 2003, Kazakhstan
- 11 Japanese Research Society for Gastric Cancer. The general rules for gastric cancer studies in surgery and pathology, 12th ed. Tokyo: 1993: 64-67
- 12 **Lauren P**. The two histological main types of gastric carcinoma diffuse and so called intestinal type carcinoma. *Acta Pathol Microbiol Scand* 1965; **64**: 31-49
- 13 **Lerner MR**, Andrews NC, Miller G, Steitz JA. Two small RNAs encoded by Epstein-Barr virus and complexed with protein are precipitated by antibodies from patients with systemic lupus erythematosus. *Proc Natl Acad Sci U S A* 1981; **78**: 805-809
- 14 **Arrand JR**, Rymo L. Characterization of the major Epstein-Barr virus-specific RNA in Burkitt lymphoma-derived cells. *J Virol* 1982; **41**: 376-389
- 15 **Clemens MJ**. The small RNAs of Epstein-Barr virus. *Mol Biol Rep* 1993; **17**: 81-92
- 16 **Chang KL**, Chen YY, Shibata D, Weiss LM. Description of an *in situ* hybridization methodology for detection of Epstein-Barr virus RNA in paraffin-embedded tissues, with a survey of normal and neoplastic tissues. *Diagn Mol Pathol* 1992; **1**: 246-255
- 17 **Preston DL**, Lubin JH, Pierce DA. Epicure user's guide. Seattle: Hirossoft International Corp 1991
- 18 **Hosmer DW**, Lemeshow S. Applied logistic regression. New York: John Wiley Sons, 1989
- 19 **Anagnostopoulos I**, Hummel M. Epstein-Barr virus in tumours *Histopathology* 1996; **29**: 297-315
- 20 **Shibata D**, Hawes D, Stemmermann GN, Weiss LM. Epstein-Barr virus-associated gastric adenocarcinoma among Japanese Americans in Hawaii. *Cancer Epidemiol Biomarkers Prev* 1993; **2**: 213-217
- 21 **Harn HJ**, Chang JY, Wang MW, Ho LI, Lee HS, Chiang JH, Lee WH. Epstein-Barr virus-associated gastric adenocarcinoma in Taiwan. *Hum Pathol* 1995; **26**: 267-271
- 22 **Qiu K**, Tomita Y, Hashimoto M, Ohsawa M, Kawano K, Wu DM, Aozasa K. Epstein-Barr virus in gastric carcinoma in Suzhou, China and Osaka, Japan: association with clinicopathologic factors and HLA-subtype. *Int J Cancer* 1997; **71**: 155-158
- 23 **Zimmer U**, Adldinger HK, Lenoir GM, Vuillaume M, Knebel-Doeberitz MV, Laux G, Desgranges C, Wittmann P, Freese UK, Schneider U. Geographical prevalence of two types of Epstein-Barr virus. *Virology* 1986; **154**: 56-66
- 24 **Boyle MJ**, Sewell WA, Sculley TB, Apolloni A, Turner JJ, Swanson CE, Penny R, Cooper DA. Subtypes of Epstein-Barr virus in human immunodeficiency virus-associated non-Hodgkin lymphoma. *Blood* 1991; **78**: 3004-3011
- 25 **Chen XY**, Pepper SD, Arrand JR. Prevalence of the A and B types of Epstein-Barr virus DNA in nasopharyngeal carcinoma biopsies from southern China. *J Gen Virol* 1992; **73** (Pt 2): 463-466
- 26 **Fukayama M**, Hayashi Y, Iwasaki Y, Chong J, Ooba T, Takizawa T, Koike M, Mizutani S, Miyaki M, Hirai K. Epstein-Barr virus-associated gastric carcinoma and Epstein-Barr virus infection of the stomach. *Lab Invest* 1994; **71**: 73-81
- 27 **Herrera-Goepfert R**, Reyes E, Hernandez-Avila M, Mohar A, Shinkura R, Fujiyama C, Akiba S, Eizuru Y, Harada Y, Tokunaga M. Epstein-Barr virus-associated gastric carcinoma in Mexico, analysis of 135 consecutive gastrectomies in two hospitals. *Mod Pathol* 1999; **12**: 873-878
- 28 **Mori M**, Sugimachi K, Ohiwa T, Okamura T, Tamura S, Inokuchi K. Early gastric carcinoma in Japanese patients under 30 years of age. *Br J Surg* 1985; **72**: 289-291
- 29 **Nakamura T**, Yao T, Niho Y, Tsuneyoshi M. A clinicopathological study in young patients with gastric carcinoma. *J Surg Oncol* 1999; **71**: 214-219
- 30 **Tso PL**, Bringaze WL, Dauterive AH, Correa P, Cohn I. Gastric carcinoma in the young. *Cancer* 1987; **59**: 1362-1365
- 31 **Kitamura K**, Yamaguchi T, Yamamoto K, Ichikawa D, Taniguchi H, Hagiwara A, Sawai K, Takahashi T. Clinicopathological analysis of gastric cancer in young adults. *Hepatogastroenterology* 1996; **43**: 1273-1280
- 32 **Lim S**, Lee HS, Kim HS, Kim YI, Kim WH. Alteration of E-cadherin-mediated adhesion protein is common, but microsatellite instability is uncommon in young age gastric cancers. *Histopathology* 2003; **42**: 128-136
- 33 **Hsieh LL**, Lin PJ, Chen TC, Ou JT. Frequency of Epstein-Barr virus-associated gastric adenocarcinoma in Taiwan. *Cancer Lett* 1998; **129**: 125-129