RESEARCH ARTICLE

Profiles of Epstein-Barr Virus Associated Gastric Carcinomas in Brunei Darussalam

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Abstract

Background: Gastric cancer is the second most common gastrointestinal cancer and is largely attributed to *Helicobacter pylori (H. pylori)* infection. In addition, studies have also shown association with Epstein-Barr virus (EBV) in 10% of gastric cancers. This study assessed the characteristics of EBV associated gastric cancers (EBVaGC) in Brunei Darussalam. Materials and Methods: This study included gastric cancers diagnosed between 2008 and 2012, registered with the Department of Pathology RIPAS Hospital, Brunei Darussalam. Clinical case notes were systematically reviewed. Histology specimens were all stained for EBV and also assessed for intestinal metaplasia and *H. pylori*. Results: There were a total of 81 patients (54 male and 27 females) with a mean age of 65.8±14.8 years included in the study. Intestinal metaplasia and active *H. pylori* infection were detected in 40.7% and 30.9% respectively. A majority of the tumors were proximally located (55.6%), most poorly differentiated (well differentiated 16%, moderately differentiated 30.9% and poorly differentiated 53.1%) and the stages at diagnosis were; stage I (44.4%), stage II (23.5%), stage III (8.6%) and stage IV (23.5%). EBV positivity (EBVaGC) was seen in 30.9%. Between EBVaGC and EBV negative gastric cancers, there were no significant differences (age, gender, ethnic group, presence of Intestinal metaplasia, tumor locations, stages of disease and degree of tumor differentiation). Conclusions: This study showed that a third of gastric cancers in Brunei Darussalam were positive for EBV, higher than what have been reported in the literature. However, there were no significant differences between EBVaGC and EBV negative gastric cancers. This suggests that the role of EBV in gastric cancer may be mostly incidental rather than any causal relation. However, further studies are required.

Keywords: Epstein-Barr virus - gastric carcinoma - *Helicobacter pylori* - prevalence - brunei

Asian Pac J Cancer Prev, **15 (23)**, 10489-10493

Introduction

Gastric cancer is the second most common gastrointestinal cancers after colorectal cancers and is the second most common cause of cancer related death (Ling et al., 1994; Zur et al., 2004). *Helicobacter pylori (H. pylori)* infection is the most common cause of gastric carcinogenesis (Lee et al., 2009) and is classified as a Class 1 carcinogen by the World Health Organization. Apart from *H. pylori* infection, other reported risk factors include pernicious anemia, environmental factors such as diet (excess nitrate and lack of dietary nutrients), smoking, alcohol intake, previous gastric surgery and *Epstein-Barr virus* infection (EBV) infection (Zur et al., 2004).

Infection with EBV, a herpes virus is common and it is estimated that 90% of the world population to have been exposed to this virus before adolescence. EBV has been associated with several neoplasms; nasophargyngeal tumor (common in the east), various lymphomas of B-cell origin (i.e. Burkitt's lymphoma, Hodgkin lymphoma), salivary gland, thymus and even stomach cancer (Uozaki and Fukayama, 2008). Burke et al. first established the association between EBV and lymphoepithelial carcinoma of the stomach (Kim, 2011). Shibata and Weiss, in 1992 later reported that EBV involvement can also been extended to a subset of ordinary gastric carcinoma (Toshikazu et al., 1998). Studies have estimated 10% of gastric cancer to be positive, and hence associated with EBV (Lopes et al., 2004; Zur et al., 2004; Akiba et al., 2008). These findings imply that EBV may play a role in the development of EBV positive gastric cancer or EBV associated gastric cancer (EBVaGC). It has been postulated that EBVaGC may not go through the same process described for *H. pylori* related gastric cancer, where it is expected to see a sequence of changes from inflammation, atrophy, intestinal metaplasia, dysplasia and carcinoma (Ling, 1994). It is believed that EBV infection drives the infected cells to be immortalized, proliferate and in some cases, progress to EBVaGC (Kim, 2011). However, the role of EBV in epithelial carcinogenesis

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is complex and multi-factorial and not fully elucidated (Tsao et al., 2014).

Similar to what have been shown in the literature, gastric cancer is also the second most common gastrointestinal cancer in Brunei Darussalam (Mohammad et al., 2014), and although it is declining (Chong et al., 2014), it remains a significant cause of morbidity and mortality. Similarly, *H. pylori* infection is the most common cause due to the high prevalence (Chong et al., 2009). To date, there is no data on EBVaGC in Brunei Darussalam. This study assesses the prevalence and characteristic of EBVaGC in Brunei Darussalam.

Materials and Methods

Patient population

This study was a retrospective based cross-sectional study. Patients diagnosed with gastric cancers were identified from the registry maintained by the Department of Pathology, the only state laboratory that handles all histological specimens, located in RIPAS Hospital, the major tertiary referral centre in Brunei Darussalam. This cancer registry captures all histology proven gastric cancers for the country.

A total of 81 consecutive patients who had been histologically diagnosed with gastric cancer from the four main hospitals in Brunei Darussalam between 1st January 2008 and 31st September 2012 were included in the study.

Clinicopathologic data collected included age, gender, types of carcinoma, location of tumor and staging at diagnosis. Histopathologic data collected included differentiation of carcinoma, presence of intestinal metaplasia, and *H. pylori*. Data collection was conducted within RIPAS Hospital only. Approval for the study was obtained from the Medical and Health Research and Ethics Committee, Brunei Darussalam.

Immunohistochemical analysis

Based on the clinical case notes and biopsy reports, all patients diagnosed with gastric cancers during the study period were studied for EBV through immunohistochemical staining. Monoclonal antibodies against latent membrane protein 1 (LMP-1) was used to detect EBV-specific proteins. The slides were then incubated in a detection kit in accordance with the manufacturer's instructions.

Histological Classification, Pathology and Staging

The tumors location was classified anatomically as cardia, fundus, body and antrum based on the predominant location of the tumors. For statistical analyses, the tumor locations were categorized into proximal (Fundus, cardia and body) and distal (antrum and pylorus). Histological diagnosis and grade of differentiation were determined in accordance with World Health Organization criteria for gastric cancer (WHO, 2000). The TNM classification was applied for staging.

Statistical analysis

Statistical analysis was done using the Statistical Package for Social Sciences (SPSS), version 20.0. Collected patient demographics, clinicopathological and histopathological data were presented in percentages and frequency. For most variables, two categories were analysed in pairs as EBV positive versus EBV negative and present versus absent. We analysed categorical variables using Pearson's Chi-square and resorted to Fisher's exact test when the assumptions for Chi-square was not met. The measure of association between variables and EBV involvement used was the odds ratio, defined as the ratio of the odds corresponding to one particular combination of factor levels divided by the odds for some other combination.

Results

Our study included 81 patients diagnosed with gastric carcinoma. Overall, gastric cancer was more common among males whereby the ethnic breakdown is consistent with the national breakdown. The mean age of patients was 65.8 ± 14.8 (Range: 25-90). The tumors weredistributed at the fundus/cardia (n=15, 18.5%), body (n=30, 37.0%) and antrum/pylorus (n=36, 44.4%), and upon diagnosis, majority were poorly differentiated (53.1%) at Stages I and II (44.4% and 22.6% respectively). Intestinal metaplasia and active *H. pylori* infections were detected in 40.7% (n=33) and 32.1% (n=25) respectively. EBV positivity (EBVaGC) was seen in a third of the patients (30.9%). The demographic and tumor characteristics are shown in Table 1.

Among the risk factors for gastric cancers, 11 patients (13.6%) were positive for both EBV and active *H. pylori* infection, eight patients (9.0%) were positive for EBV and IM and three patients were positive for all of EBV, *H. pylori* and IM.

Comparisons between EBVaGC and non EBVaGC showed no significant differences apart from more active *H. pylori* infection among EBVaGC. There was no difference in the mean age between the two groups (EBVaGC 65.1±15.9 *vs* non EBVaGC 66.1±14.5 years old, p=0.853). The comparisons are shown in Table 2.

Table 1. Sociodemographic Characteristics of Study Sample

Subjects		Mean (Standard deviation)	N (%)
Age (year)		65.8 (14.8)	
Gender	Male		54 (66.7)
	Female		27 (33.3)
Ethnicity	Malay		68 (84.0)
	Chinese		9 (11.1)
	Others		4 (4.9)
Tumor			
Location	Proximal		44 (54.3)
	Distal		37 (45.7)
Degree of differentiation			
	Well		13 (16.0)
	Moderately		25 (30.9)
	Poorly		43 (53.1)
Stage	I / II		36 (44.4)/ 19 (23.5)
	III / IV		7 (8.6)/ 19 (23.5)
Intestinal metaplasia (Positive)			33 (40.7)
Active *H. pylori* infection (Positive)			25 (30.9)
EBV Positive (EBVaGC)			25 (30.9)

SD= Standard Deviation; Freq. =Frequency

Table 2. Association between Clinicopathological Variables and EBV Involvement in Gastric Cancer

Variable	EBVaGC n (%)	Non-EBVaGC n (%)	P value
Mean age	65.1±15.6	66.1±14.5	0.838
Gender			
Male	17 (68.0)	37 (66.1)	0.865
Female	8 (32.0)	19 (33.9)	
Race			
Malay	23 (92.0)	45 (80.4)	0.083
Chinese	0 (0.0)	9 (16.1)	for trend
Others	2 (8.0)	2 (3.6)	
Tumor			
Location			
Proximal	13 (52.0)	31 (55.4)	0.779
Distal	12 (48.0)	25 (44.6)	
Degree of differentiation			
Well	6 (24.0)	7 (12.5)	0.114 for trend
Moderately	4 (16.0)	21 (37.5)	
Poorly	15 (60.0)	28 (50.0)	
Stage of disease			0.49
I	15 (53.6)	23 (41.1)	for trend
II	7 (25.0)	12 (21.4)	
III	2 (7.1)	5 (8.9)	
IV	4 (14.3)	16 (28.6)	
Intestinal metaplasia			
Positive	8 (32.0)	25 (44.6)	0.285
Negative	17 (68.0)	31 (55.4)	
Active H. pylori infection			
Positive	11 (44.0)	14 (25.0)	0.087
Negative	14 (56.0)	42 (75.0)	

Discussion

Similar to what have been reported in most countries (IARC, 2012), gastric cancer is the second most common gastrointestinal cancer in Brunei Darussalam (Mohammad et al., 2014). Unlike the association with *H. pylori* which have been well studied, there are fewer studies on the association between EBV and gastric cancer. To date; there has been no study from Brunei Darussalam looking at EBV and gastric cancer. Our study showed that the estimated point prevalence of EBVaGC was 30.9%%, much higher than rates reported from other countries, generally around 10% of all gastric cancer (Lopes et al., 2004; Zur et al., 2004; Akiba et al., 2008).

EBVaGC has been reported to differ from non-EBV gastric cancer. Compared to non EBV gastric cancer, EBVaGC is characterized by male predominance, younger age at a diagnosis, propensity for multifocal occurrence, higher incident in gastric remnant recurrence, higher lymphocytes infiltrations and also the degree of differentiation. The pathogenesis of EBVaGC is also reported to differ from *H. pylori* related gastric cancer which typically goes through the atrophic, metaplasia, and dysplasia sequence (Uemera et al., 1994). Infection with EBV has been shown drives infected gastric cell, resulting in changes that can eventually lead to development of gastric cancer (EBVaGC). Many molecular changes such as DNA hypermethylation, role of EBV encoded miRNA and viral induced oncoproteins have been studied, but the exact pathogenesis is not yet fully understood (Iizasa et al., 2012; Lung et al., 2013). A recent genome wide transcriptome study identified many alterations in genes, gene expression, and gene methylation that affect different signalling networks (Laing et al., 2014).

In our study, we did not observe any significant differences between EBVaGC and non EBV gastric cancer, either in the patients demographic or tumors characteristics. However, there were some minor differences.

We also found that there was male predominance with a ratio of almost 2:1. Almost all studies have shown male predominance in the expression of EBV (Roukos et al., 2002; Camtu, 2009). A large Dutch cohort study reported a much higher male to female ratio of 9.8 (Van et al., 2004). This preponderance is not unexpected considering that gastric cancer is more common in men. Apart from the obvious gender difference, other risk factors include lifestyle and occupational factors, intrinsic biological and and hormonal factors (Roukos et al., 2002).

The mean age for EBVaGC was 62.9±17.1SD, no difference compared with non-EBVaGC (66.0±14.8SD). This is in contrast with what have been reported, younger age for EBVaGC compared to the non EBV cancer. A Japanese study reported that patients with EBVaGC tended to be older siblings, whereas another study reported the opposite. However, the duration of EBV infections may be an important factor (Koriyama et al., 2005; Campos et al., 2006).

By racial distribution, the majority of EBVaGC in our study was among Malays (n=34, 41.5%). This is consistent with population breakdown with the Malays being the predominant population. There was a trend towards significance (p=0.083 for trend) with slightly more Malays and fewer Chinese affected by EBVaGC. A study from Malaysia (Karim and Pallesen, 2003), a country which has almost similar population demographic, reported that 10% positivity for EBV in patients with gastric cancers, majority (80%) among Chinese and non among the Malays. However, this study only included a small number of cases (n=50) and included a very few Malays (Chinese 27, Indian 19 and Malays 4). Given the small sample sizes of ours and the Malaysian study (Karim and Pallesen, 2003), it is uncertain if there is any actual difference between the different ethnic groups. Therefore, further studies with larger sample size are required.

Among the tumor characteristics studied, there were also no difference between tumor site, degree of differentiation, stage of disease at diagnosis, presence of intestinal metaplasia and presence of active *H. pylori* co-infection. Proximal tumor, defined as tumor of the body, cardia and fundus were equally common between EBVaGC and non EBV cancer. However, with regard to the degree of differentiation, there were more EBVaGC with moderate degree of differentiation and fewer of the well and poorly differentiated tumors. However, the difference was small and non-statistically significant. Similarly, this was also reflected in the tumor staging at diagnosis with no differences observed. We did not formally assess and report the degree of lymphatic infiltration. However, earlier cases showed more lymphocytic infiltrations consistent with what have been reported in the literature. A recent large multi-center study reported that EBVaGC tended to have lower tumor-node-metastasis based on the

TNM staging system and EBV positivity was associated with better survival (Camargo et al., 2014).

Although, studies have shown that EBV lead to molecular changes resulting in gastric carcinogenesis, different to that of *H. pylori* sequence, and that there are certain characteristics that are unique to EBVaGC, there are still differences and conflicting results based on studies from other populations. This suggests that EBVaGC is a spectrum where in some cases, the main driving agent for carcinogenesis is the EBV itself in patient with susceptibility whereas in the rest of the cases, EBV maybe a bystander or is only contributory and other agents, either environmental and genetics (predisposing or protective) are the main determining factors (Kaung et al., 2014; Malakar et al., 2014; Tuncel et al., 2014). High salt intake is a proven risk factor and high salt intake is also common in our population. Smoking has also been shown to have an impact; more on EBV related than non EBV related gastric cancers (Camargo et al., 2014). *H. pylori* infection is a common infection among our population (Chong VH et al., 2009) and this may attenuate the impact of EBV on gastric carcinogenesis. Other differences such as the genetic make-up of our predominant Malay population, a population that remains not well studied may be important.

There are a number of limitations with our study. First, being a retrospective study is inherently associated with certain limitations such non-standardised data recording or incomplete data. Second, the sample size was small but being a nation with a small population, the sample size was representative of the entire population. Finally, we had only used active *H. pylori* and did not consider those that may have been previously treated. Addition of serum serology to indicate exposure may increase the number of *H. pylori* cases. Even after eradication, the changes associated with *H. pylori* infection in particular mucosal atrophy and intestinal metaplasia may progress and lead to gastric cancer (Wong et al., 2004). The main strength of our study was that all the cases were captured by one centre and the techniques for staining for EBV was standardised.

In conclusion, our study showed that a third of gastric cancers in Brunei Darussalam were positive for EBV, higher than what have been reported in the literature. However, there were no significant differences in the profiles between EBVaGC and EBV negative gastric cancers in our population with predominant Malay population. This is in contrast to widely accepted characteristics of EBVaGC. This suggests that EBVaGC may be different between populations where the various environmental and genetic factors are different.

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