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ORIGINAL ARTICLE

Distribution of gastric adenocarcinoma subtypes in different ethnicities in Kuala Lumpur, Malaysia

Asif SUKRI, Alfizah HANAFIAH, Nik Ritza KOSAI*, Mustafa Mohamed TAHER* and Isa MOHAMED ROSE**

*Department of Medical Microbiology and Immunology, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Cheras, Kuala Lumpur, Malaysia, *Department of Surgery, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Cheras, Kuala Lumpur, Malaysia, **Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Cheras, Kuala Lumpur, Malaysia*

Abstract

The multiracial population in Malaysia has lived together for almost a century, however, the risk of gastric cancer among them varies. This study aimed to determine the distribution of different gastric adenocarcinoma subtypes and *Helicobacter pylori* infection status among gastric adenocarcinoma patients. Patients with gastric adenocarcinoma were enrolled from November 2013 to June 2015. Blood samples were collected for detection of *H. pylori* using ELISA method. Gastric adenocarcinoma cases were more prevalent in the Chinese (52.8%), followed by the Malays (41.7%) and least prevalent in the Indians (5.6%). Gastric adenocarcinoma located in the cardia was significantly more prevalent in the Malays (66.7%) compared to the Chinese (26.3%), whereas non-cardia cancer was diagnosed more in the Chinese (73.7%) compared to the Malays (33.3%) [$P = 0.019$; OR = 5.6, 95 CI: 1.27 to 24.64]. The Malays also had significantly higher prevalence of gastric tumour located at the cardia or fundus than other gastric sites compared to the Chinese ($P = 0.002$; OR: 11.2, 95% CI: 2.2 to 56.9). Among the cardia gastric cancer patients, 55.6% of the Malays showed intestinal histological subtype, whereas all the Chinese had the diffuse subtype. More than half of the patients (55.3%) with gastric adenocarcinoma were positive for *H. pylori* infection and among them, 66.7% were Chinese patients. The risk of gastric adenocarcinoma in our population is different among ethnicities. Further studies on host factors are needed as it might play an important role in gastric cancer susceptibility in our population.

Keywords: cardia gastric cancer, non-cardia gastric cancer, intestinal-type, diffuse-type, *Helicobacter pylori*

INTRODUCTION

Although the incidence of gastric cancer has decreased worldwide in recent decades, it is still the third leading cause of cancer mortality and major problem in developing countries.[1-3] A decline in incidence of gastric cancer has been attributed to increased food refrigeration and consumption of more fresh fruits and less salty foods.[4] The factors that are reported to increase risk of gastric cancer include gender[1], *H. pylori* infection, high consumption of certain diets such as miso soup and salty foods[5], smoking habit[5,6] and host genetic factors.[7-10] In Peninsular Malaysia, gastric cancer is the seventh and the tenth most common cancer in males and females, respectively. The prevalence of gastric cancer in Malaysia has been disproportionate among the three major ethnicities of Malays, Chinese and Indians. Overall, the incidence of gastric cancer cases is highest in the Chinese (66%) followed by the Malays (18.7%) and lowest in the Indians (15.3%).[11]

Anatomical subsite of gastric cancer has been categorised according to the WHO classification into cardia or non-cardia gastric cancers. Cardia gastric cancer arises in the area of the stomach adjoining the oesophageal-gastric junction, whereas non-cardia gastric cancer arises from more distal regions of the stomach.[12,13] Non-cardia subsites were considered to include the fundus,

Address for correspondence: Alfizah Hanafiah, Department of Medical Microbiology and Immunology, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Cheras, Kuala Lumpur, Malaysia. Tel: +603-91459539; Fax: +603-91459524. E-mail: alfizah@ppukm.ukm.edu.my

corpus, antrum, pylorus, lesser and greater curvatures. Non-cardia cancers show a strong positive association with *H. pylori* infection, whereas cardia cancer has negative, positive or no association with *H. pylori* infection.[14] Non-cardia cancer is the result of progression from *H. pylori* superficial gastritis to atrophic gastritis and hypochlorhydria to dysplasia and finally to cancer.[14] The aetiology of cardia gastric cancer remains poorly understood and different from those of the rest of the stomach. One reason for this may be the anatomical complexity of the cardia. The cardia mucosa extends from the oxyntic mucosa of the body of the stomach to the squamous mucosa of the distal oesophagus. The mucosa consists of columnar mucosa resembling that of the gastric antrum. Cardia gastric cancer incidence rates are typically lower than non-cardia gastric cancer.[12]

Histologically, two distinct variants of gastric cancer have been identified: diffuse-type and intestinal-type gastric adenocarcinoma. The pathogenesis of intestinal type of gastric cancer involves a multi-stage process that is initiated by the transition from normal mucosa to chronic superficial gastritis, atrophic gastritis, intestinal metaplasia, dysplasia and finally adenocarcinoma[15,16] while the diffuse type is a familial disease that has been associated with mutation in the *E-cadherin* gene.[17] The diffuse-type has similar frequencies in all geographic locations whilst, the intestinal-type shows variable incidence rates in different geographical regions.[18]

H. pylori is a gram negative, microaerophilic, oxidase- and urease-positive bacteria that is able to colonize the human stomach and infect half of the world population.[19] The discovery of *H. pylori* had been met with scepticism since it was believed no organism was able to live in the acidic human stomach.[20] The seroprevalence percentage of *H. pylori* infection in Malaysia is 35.9%, with the Indian ethnic having the highest rate followed by the Chinese and Malays.[21,22] The low incidence of gastric cancer cases among ethnic Indians inspite of high infection rate with *H. pylori* has been considered as the Indian paradox.[23] There is a scarcity of data regarding the distribution of anatomical and histological subtypes of gastric cancer together with the status of *H. pylori* infection among gastric cancer patients in the Malaysian population. Therefore, this study was undertaken to compare the distribution of anatomical subsites and histological subtypes of gastric adenocarcinoma among patients of different ethnic groups in a tertiary hospital in Kuala Lumpur, Malaysia and also to determine the *H. pylori* infection status among gastric adenocarcinoma patients.

MATERIALS AND METHODS

Patient population and samples

Patients who had been clinically diagnosed with gastric adenocarcinoma at Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Kuala Lumpur, Malaysia from November 2013 till June 2015 were consecutively recruited to participate in this study. Information regarding the study was explained prior to enrolment and informed consent was obtained from each patient. This study was approved by the Research Ethics Committee of UKMMC (UKM 1.5.3.5/244/02-01-02-SF0958). The biographic data of patients including age, gender and ethnicity were documented.

Patients included in the study were those who had undergone gastrectomy or gastroscopic examination. A total of eight gastric biopsies of the tumour were taken from patients who underwent gastroscopic examination. Gastric tissues (from gastrectomy) and biopsies were sent to the Department of Pathology, UKMMC for histopathological examination. Further information as to the type of gastric cancer, the site of tumour and its histological type according to Lauren classification were determined.[24] Three mL of venous blood samples were collected from patients and centrifuged for serum collection. Sera were stored at -20ºC until used for serological test. Patients who already received neo-adjuvant chemotherapy were excluded from *H. pylori* serological test to minimize the effect of seroreversion towards *H. pylori* infection.[25]

Serological test for H. pylori

H. pylori IgG antibody concentration was determined serologically by ELISA kit (Arigo Bio, Hsinchu City, Taiwan) which has functional sensitivity of 1.16 U/mL. Briefly, sera were diluted to 1:100, controls and calibrators were added to 96-well plates coated with *H. pylori* antigens. Plates were incubated and washed for three times. Antibody solution was added, incubated and washed followed by addition of TMB reagent and stop solution. Each test was performed in triplicate. Optical density was read at 450 nm. The results were interpreted as positive, intermediate or negative when the IgG concentration was more than 12 U/mL, 8-12 U/mL or less than 8 U/mL, respectively.

Statistical analysis

Comparisons of gastric cancer cases for *H. pylori* infection, histopathology and ethnicity were made using Pearson's chi square or Fisher's exact test, whenever appropriate. A *P* value less than 0.05 was considered as significant. All statistical analyses were performed using SPSS version 21 software. Each sample for ELISA test was performed in triplicate and calculated as mean ± standard deviation.

RESULTS

Patient demography and tumour characteristic

A total of 36 gastric adenocarcinoma patients were included in the study comprising of 15 (41.7%) Malays, 19 (52.8%) Chinese and two (5.6%) Indians. There were 26 males (72.2%) and 10 females (27.8%) ranging from 30 to 81 years old, with a mean age of 61.6 ± 14.5 years (median age = 64 years). The male-to-female ratio was 2.6.

Gastric adenocarcinoma had different topographical origin, namely cardia and non-cardia gastric cancers. Fifteen (41.7%) patients had tumours occurring at the cardia/gastroesophageal junction site (GEJ) while 21 (58.3%) patients had tumours from non-cardia sites. Of non-cardia site tumours, nine (42.9%) tumours were located at the pylorus, six (28.6%) tumours at the body, four (19%) at the fundus and one tumour (4.8%) was located at antrum. One patient (4.8%) had tumours located at body and pylorus.

The male patients had almost similar distribution of cardia and non-cardia cancers being 46.2% (12/26) and 53.8% (14/26), respectively. In contrast, the majority of female patients (70%; 7/10) had non-cardia cancers compared to cardia cancers (30%; 3/10).

For the analysis of gastric cancer distribution among different ethnicities, Indian patients were not included because only two Indian patients were diagnosed with gastric adenocarcinoma during the study period. Both patients had non-cardia cancer. Table 1 summarizes the distribution of tumour location between the Malays and Chinese. The Malays had a significantly higher prevalence of cardia cancer (66.7%; 10/15) compared to the Chinese (26.3%; 5/19), whereas non-cardia gastric cancer was diagnosed more in the Chinese (73.7%; 14/19) compared to the Malays (33.3%; 5/15) (Pearson's chi square, $P = 0.019$; odds ratio = 5.6, 95% CI: 1.27 to 24.64). Upon stratification of results based on the occurrence of tumours at cardia or fundus *versus* other gastric sites, we found that the Malays (80%; 12/15) had more significantly pronounced higher occurrence of tumour at cardia or fundus site compared to the Chinese (26.3%; 5/19) (Pearson's chi square, $P = 0.002$; odds ratio = 11.2, 95% CI: 2.2 to 56.9).

Histological subtypes of gastric adenocarcinoma using Lauren's classification showed that 41.8% (14/34) of tumours were intestinal subtype, 52.9% (18/34) were diffuse subtype, and 5.9% (2/34) were mixed subtype. Figure 1 shows the histopathological features of the different subtypes of gastric adenocarcinoma. Gastric cancer classification for two cases were not available because of insufficient size of the biopsy specimen. Among the Malays; intestinal, diffuse and mixed types of gastric cancers were identified in 40% (6/15), 53.3% (8/15) and 6.7% (1/15), respectively. The distribution of histological subtypes of gastric cancer among the Chinese were 35.3% (6/17) intestinal subtype, 58.8% (10/17) diffuse subtype and 5.9% (1/17) mixed subtype. The two Indian patients had intestinal subtype of gastric cancer.

TABLE 1: The distribution of gastric adenocarcinomas by tumour location between Malay and Chinese patients

Tumour location	Ethnicity, n (%)		*P* value
	Malay (n = 15)	Chinese (n = 19)	
Cardia	10 (66.7)	5 (26.3)	0.019
Non-cardia	5 (33.3)	14 (73.7)	
Cardia/fundus	12 (80)	5 (26.3)	0.002
Other sites	3 (20)	14 (73.3)	

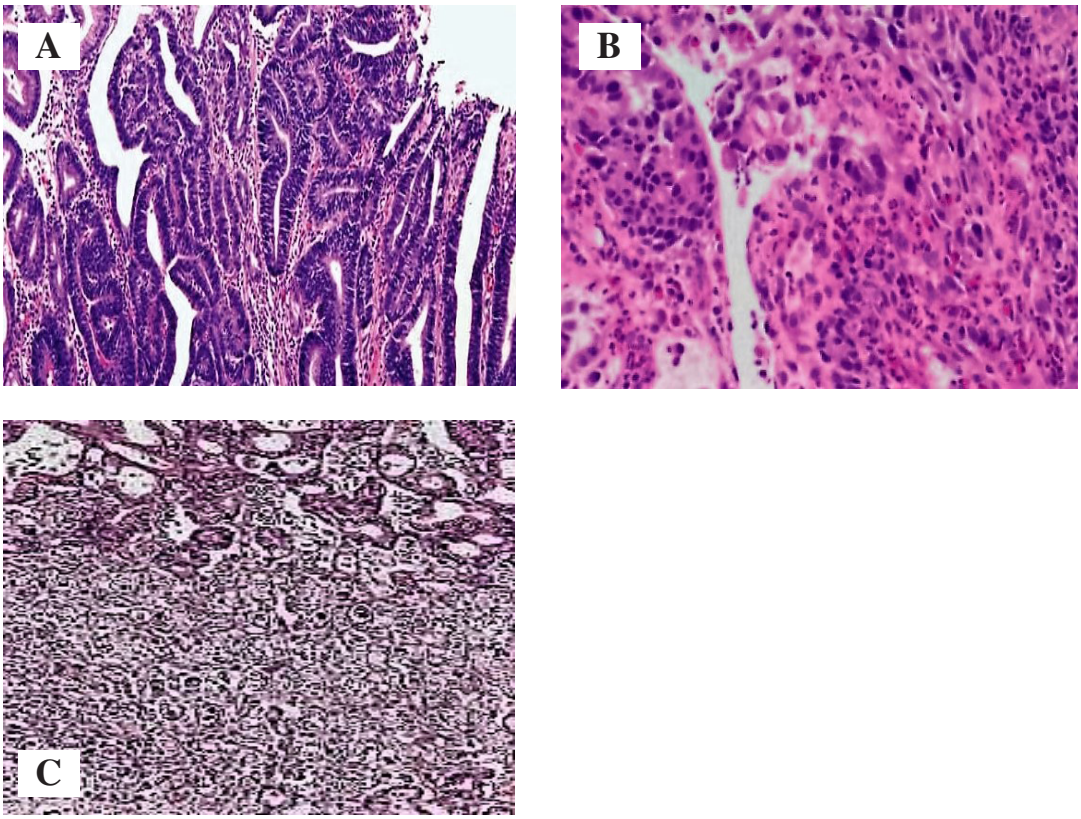

FIG. 1: Histopathological features of the different subtypes of gastric adenocarcinomas. A) Intestinal subtype (sample from patient no. G9), B) Diffuse subtype (sample from patient no. G10), and C) Mixed subtype (sample from patient no. G2)

The cardia and non-cardia cancers had similar distribution of histological subtypes. Of the 14 cardia cancers, five (35.7%) were intestinal, eight (57.1%) were diffuse and one (7.1%) was of mixed subtype. The 20 non-cardia cancers comprised nine (45%) intestinal, 10 (50%) diffuse and one (5%) mixed subtypes. Among the Malays, 80% of patients with non-cardia cancer had diffuse subtype and 55.6% of patients with cardia cancer had intestinal subtype (Table 2). However, no significance difference was observed (Fisher's exact test, $P = 0.301$). All the Chinese patients with cardia cancer had diffuse subtype whereas Chinese patients with non-cardia cancer had similar percentage of intestinal and diffuse subtypes (Fisher's exact test, $P = 0.234$).

H. pylori infection status in gastric cancer patients

Among the 22 gastric adenocarcinoma patients who did not receive chemotherapy, 16 agreed to give a blood sample for determination of *H. pylori*

TABLE 2: The distribution of gastric adenocarcinomas according to histological subtypes between Malays and Chinese

Ethnic/cancer type	Intestinal, n (%)	Diffuse, n (%)
Malays:		
Cardia (n = 9)	5 (55.6)	4 (44.4)
Non-cardia (n =5)	1 (20.0)	5 (80)
Chinese:		
Cardia (n = 4)	0	4 (100)
Non-cardia (n= 12)	6 (50)	6 (50)

sero-status. Blood was not taken from other 14 patients because these patients had undergone chemotherapy. Of the 16 patients for whom *H. pylori* infection status were tested, five were Malays, nine Chinese and two Indians. Overall, 56.3% (9 of 16) of gastric cancer patients were positive for *H. pylori* infection. Further analysis on the *H. pylori* infection status were carried out based on the percentage of positivity (statistical analysis was not done) due to small numbers of the samples.

H. pylori positive was detected in 66.7% (6 of 9), 20% (1 of 5) and 100% (2 of 2) of the Chinese, the Malays and the Indians, respectively. The majority of male patients (63.6%; 7 of 11) were infected with *H. pylori* compared to the females (40%; 2 of 5). Concentration of IgG antibody in *H. pylori*-positive and *H. pylori*-negative gastric cancer patients ranged from 14-165 U/mL and 4-7.5 U/mL, respectively. The mean value of IgG antibody in *H. pylori*-positive patients was 107.3 ± 50.9 U/mL. Six of nine (66.7%) *H. pylori*-positive patients had IgG antibody concentration higher than 100 U/mL. Four (66.7%) of six patients with IgG titer higher than 100 U/mL were Chinese while the other two (33.3%) were an Indian and a Malay.

We also analysed the *H. pylori* infection status among patients with different tumour location and histological subtypes. *H. pylori* infection was detected in 50% (3 of 6) and 60% (6 of 10) of patients with cardia and non-cardia gastric cancer, respectively. *H. pylori* infection was detected in 54.5% (6 of 11) and 60% (3 of 6) of patients diagnosed with intestinal and diffuse subtypes, respectively.

DISCUSSION

Malaysia is a multicultural, multiethnic country in which Malay, Chinese and Indian ethnic groups make up the main races. Based on updated 2010 population consensus, Malay and aboriginal ethnics make up 67.4% of Malaysian population, followed by Chinese (24.7%), Indians (7.3%) and others (0.7%).[26] Even though the multiple ethnic groups in Malaysia have lived together for two generations, we found that the distribution of gastric cancer among ethnicities was disproportionate. The Chinese had the highest percentage occurrence of gastric cancer followed by the Malays and the Indians. The results were concordant with previous reports.[11] The mean age of gastric cancer patients in our study was 61.6 ± 14.5 years which further confirmed that gastric cancer is a disease of the elderly. A higher frequency of gastric cancer occurrence in males compared to females in our study was consistent with findings from previous studies where gastric cancer was found to have a male preponderance.[27]

In this study, we asked whether there was any distinction between anatomical location of gastric tumour and ethnicity of patients. A difference between occurrence of cardia and non-cardia locations of gastric cancer among the Malays and the Chinese was observed in our study. Cardia gastric cancer was significantly more prevalent in the Malays, whereas non-cardia cancer was prevalent in the Chinese with odds ratio of 5.6. Additional analysis on the location of gastric tumour based on occurrence at upper stomach (cardia or fundus) site with lower anatomical sites of the stomach (body, antrum and pylorus) revealed significantly higher prevalence of gastric cancer occurrence at cardia or fundus in the Malays ($P = 0.002$) compared to the Chinese with odds ratio of 11.2. The high prevalence of cardia cancer among the Malays has been reported in an area of Malay majority in Malaysia, i.e., Kelantan state.[28] In addition, the Chinese population in Malaysia can be categorized as a high risk population to develop distal gastric cancer. The results were concordant with what has been shown in the past in that the high risk population has a susceptibility to develop distal gastric cancer compared to proximal gastric cancer.[12] Further distinct histological subtypes of cardia cancer were observed among patients of different ethnicities. The Malays had intestinal histological subtype and the Chinese had diffuse subtype. However, the difference observed in this study was not statistically significant. This might be hampered by the small number of samples analysed with stratification between anatomical sites of cancers and the histological subtypes among the Malays and the Chinese. Further study with a larger number of samples is needed to confirm this observation. Interestingly, the high percentages of cardia cancer with intestinal histological subtype in the Malays and diffuse subtype in the Chinese have not been reported before. Because prognosis of cardia cancer is poorer compared to non-cardia cancer, this finding will be important to shed light on prognosis of gastric cancer patients in our centre based on patient's ethnicity.[29]

Cardia cancer of the intestinal histological subtype is likely to be aetiologically similar to non-cardia cancer and to have arisen from original gastric mucosa if the cancerous

process reveals atrophic gastritis. In contrast, if the patient has a healthy non-atrophic gastric mucosa, then cardia cancer of the intestinal histological subtype is likely to be of aetiology similar to oesophageal adenocarcinoma and have arisen from metaplastic oesophageal mucosa produced by gastroesophageal reflux. Cardia cancers of the diffuse histological subtype are likely to be gastric in origin which consists of individually infiltrating neoplastic cells that do not form glandular structures.[14] However, in this study we cannot suggest any aetiological subtypes of cardia cancer due to the lack of information regarding the presence of atrophic gastritis among patients. An inverse relationship has been suggested between *H. pylori* infection and the risk of esophageal adenocarcinoma.[30] A low prevalence of *H. pylori* infection have been reported among the Malays.[31] A high percentage of cardia cancers among the Malays may not suggest an aetiological similarity to oesophageal carcinoma due to the low incidence of oesophageal carcinoma reported among the Malays.[32,33] The significant occurrence of adenocarcinoma located at the cardia or fundus in the Malays compared to the Chinese warrants further evaluation. Furthermore, *H. pylori* infection is detected more in non-cardia than cardia gastric cancers.

In the present study, only 56.3% of the gastric cancer patients were positive for *H. pylori* infection. The result was expected as the majority of patients enrolled in this study were elderly and most of them were diagnosed with advanced gastric cancer. This condition increases the chance of clearance of the bacteria because *H. pylori* does not colonize areas with cancer, intestinal metaplasia or atrophy and with the development of advanced gastric disease, the organism can be lost from the stomach.[34] Furthermore, *H. pylori* status is assessed only by serology after the diagnosis of cancer, underestimating the magnitude of the association between *H. pylori* and gastric cancer as a result of loss of infection.[34] A stronger *H. pylori*-gastric cancer association has been found usually in younger patients or patients with early gastric cancer.[25] We found a similar trend of *H. pylori* infection among different ethnicities and gastric cancer types as previously reported.[23,35]

Studies have shown that *H. pylori* increases the risk of developing non-cardia gastric cancer.[25,36,37] In our study, we found almost a similar proportion of patients with cardia and non-cardia cancers were infected with *H. pylori*. This may be explained in that *H. pylori* might protect the host from developing cardia gastric cancer but it might not play significant role in development of non-cardia gastric cancer.[37] Interestingly, our findings showed that the Malays had a higher rate of intestinal gastric cancer compared to the Chinese. Intestinal type cancer has been associated with *H. pylori* infection[15] while diffuse type has been associated with genetics.[17] However, our result shows that the Malays had a low prevalence of *H. pylori* infection compared to the Chinese. The contributing factors involved might be the bacterial strain-specific and the host factors. Therefore, further studies to determine the role of bacterial strain-specific and host factors in the development of different histological types of gastric cancers in the future is needed as the information is lacking.

In this study, we found that 66.7% of *H. pylori*-infected gastric cancer patients were from the Chinese ethnic. The finding is concordant with a previous report even though the composition of the study population is quite different.[23] Gastric cancer samples in the study were collected largely from Indian and Chinese populations while in our study, more samples were collected from the Malays and Chinese. Analysis of the *H. pylori* IgG concentration showed that six patients had IgG titer more than 100 U/mL and majority of them were from the Chinese patients. Higher IgG concentration corresponding to higher bacterial colonization has been demonstrated more severe inflammatory effects in *H. pylori*-infected patients.[38,39] The inability of Chinese patients to clear or suppress bacterial colonization in the stomach should be investigated further since this aspect may influence host susceptibility to gastric cancer. It has been suggested that host immune factors might be more paramount in clearing or suppressing bacterial colonization density.

In conclusion, we have revealed ethnic-related difference in tumour anatomical location in gastric adenocarcinoma Malaysian patients. In addition, the histological subtypes of cardia cancers also show ethnic-specific differences even though the difference is not statistically significant due to small number of cases in each category. This may indicate different pathogenesis of gastric cancers between the two ethnic groups (Malays and Chinese). The recognition of these two different types of gastric carcinoma among different ethnic groups may facilitate future studies and promote understanding of pathogenesis of the disease. Even though the role of *H. pylori* in gastric cancer

development cannot be disputed, further studies with larger numbers of gastric cancer cases are required to elucidate the contribution of *H. pylori* strains in the development of different gastric cancer subtypes in different ethnic groups.

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