

AB-PAS as a potential alternative for the diagnosis of intestinal metaplasia.

Sheng Quan^{1,2#}, Jinping Lin^{1,2#}, Zhinong Jiang³, Xingkang He^{1,2}, Chen Sun^{1,2}, Zhenghua Lin^{1,2}, Tingting Su^{1,2}, Shujie Chen^{1,2*}, Jianmin Si^{1,2*}

¹Department of Gastroenterology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang Province, PR China

²Institute of Gastroenterology, Zhejiang University, Hangzhou, Zhejiang Province, PR China

³Department of Pathology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang Province, PR China

#These authors contributed equally to this work

Abstract

Background: This study was to investigate the role of AB-PAS in the diagnosis of Intestinal Metaplasia (IM) as compared to HE staining, and to evaluate the relationship between *Helicobacter pylori* (*H. pylori*) infection and IM to explore whether IM was a possible pathogenic mechanism of *H. pylori* in gastric carcinogenesis.

Methods: Patients who suffered from Chronic Atrophic Gastritis (CAG) to Gastric Cancer (GC) were recruited, and the gastric tissues (CAG tissues and cancer tissues) were collected for HE staining, AB-PAS and silver staining for detection of IM and *H. pylori* infection.

Results: Twenty-six patients meeting the inclusion criteria were recruited into the present study and a total of fifty-two slices were available. Thirty-four slices were IM (+) in HE staining, while forty-three slices were IM (+) in AB-PAS. The correlation coefficient between *H. pylori* infection and IM was about 0.41 (P<0.05).

Conclusions: AB-PAS is superior in the diagnosis of IM to HE staining and IM might be one of pathogenic mechanisms underlying the *H. pylori* induced gastric carcinogenesis.

Keywords: Intestinal metaplasia, HE staining, AB-PAS, *H. pylori* infection.

Accepted on March 14, 2017

Introduction

Gastric Cancer (GC) is one of the leading causes of human cancer-related death and has the second highest incidence and morbidity globally according to the WHO statistics. The overall five-year survival rate of Early Gastric Cancer (EGC) is higher than 90% while the prognosis of Advanced Gastric Cancer (AGC) is still tremendously poor [1]. Therefore, early detection of precancerous lesions is pivotal for the prevention of GC. Intestinal Metaplasia (IM), one of precancerous lesions of GC with the nature of metaplasia [2], can be halted and reversed if interventions are given timely [3]. Hematoxylin-Eosin staining (HE staining) is the most common method used in the diagnosis of IM. However, the procedures and evaluation criteria of HE staining are complex. Thus, to investigate potential staining methods with simple procedures and evaluation criteria is necessary and urgent for the diagnosis of IM.

AB-PAS is a dyes acidic protein secreted by IM cells. Until now, few studies have reported its application in the diagnosis

of IM. This study was undertaken to investigate the role of AB-PAS in the diagnosis of IM as compared to HE staining.

There are some risk factors for GC such as family medical history, lifestyle, bile reflux, and *Helicobacter pylori* (*H. pylori*) infection [4]. Marshall and Warren originally discovered *H. pylori* in 1982. *H. pylori*, prevalent worldwide [5,6], belongs to gram negative, non-spore forming spiral bacteria, and colonizes the human stomach. It is closely related to peptic ulcer and chronic gastritis, which is also one of carcinogenic factors [7-10]. At present, the gastric carcinogenic mechanisms of *H. pylori* mainly include gene instability, cytokine, immunity, proliferation and apoptosis of gastric epithelial cells [11-14]. It also assumes that *H. pylori* accounts for the IM of gastric epithelium resulting in GC [15,16], but evidence is still conflicting. Thus, this study was to investigate the role of AB-PAS in the diagnosis of IM as compared to HE staining, and to evaluate the relationship between *H. pylori* infection and IM to explore whether IM was a possible pathogenic mechanism of *H. pylori* in gastric carcinogenesis.

Material and Methods

Compliance with ethics guidelines

All procedures followed were in accordance with the ethical standards of Institutional Review Board of Sir Run Run Shaw Hospital and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Clinical samples

A total of 5475 patients were diagnosed with GC in the Sir Run Run Shaw Hospital from January 1994 to March 2013. Inclusion criteria (diagnosed by pathology) were as follows: (i) a prior history of CAG with or without IM before being diagnosed with GC, (ii) GC was diagnosed based on specimen from the same location where CAG was previously diagnosed. Exclusion criteria were as follows: (i) patients were for the first time diagnosed with GC, (ii) gastric tissues were unavailable (iii) patients was diagnosed with signet-ring cell carcinoma, (iv) other situations not in accordance with the inclusion criteria were present.

Finally, 26 out of 5475 patients met the inclusion criteria and recruited into this study. CAG tissues and cancer tissues were collected by endoscopic biopsy and/or surgery at different time points. Three slices were obtained from each tissue for HE staining and AB-PAS for the evaluation of IM, and for silver staining for the detection of *H. pylori* infection. The flow chart is shown in Figure 1.

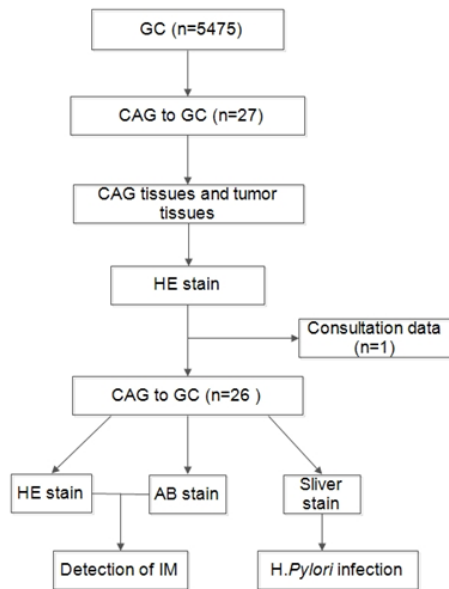


Figure 1. Flow chat of this study.

Reagents

Reagents used for different staining were as follows: (i) Hematoxylin solution (H9627-25G, lot#041M0014v) and 1% Eosin Y disodium salt (E6003-25G, lot#MKBP6048V) for HE staining, (ii) Alician Blue 8GX (SIGMA, lot#SLBB1776V) for

AB-PAS, and (iii) sliver nitrate (SIGMA S6506-25G, lot#MKBF1627) for silver staining.

Analysis and statistics

The diagnosis of IM was performed independently by three experienced pathologists according to the diagnostic criteria of Chinese Anti-Cancer Association 1998. Any disagreement was resolved by discussion. *H. pylori* infection (+) was defined when *H. pylori* was present in one and/or two slice(s). Statistical analysis was performed using SPSS version 17.0 statistical software (Chicago, IL, USA). Correlation was assessed by the Spearman rho correlation analysis. A value of $P < 0.05$ was considered statistically significant.

Results

Twenty-two male and four female patients aged 41-84 years met the inclusion criteria and were recruited into this study. Nineteen out of twenty-six lesions were from the gastric antrum and five from gastric fundus or body. The main pathologic type of GC was adenocarcinoma, and two patients were diagnosed with gastric stump carcinoma (Table 1).

Two gastric tissues (one CAG tissue and one GC tissue) were obtained from each patient, and a total of fifty-two tissues were collected. Correspondingly, there were fifty-two slices for different stainings. By HE staining, thirty-four slices were IM (+), all of which were also IM (+) by AB-PAS. However, nine out of eighteen slices negative for IM by HE staining were IM (+) by AB-PAS. In total, 43 out of 52 slices were IM (+) by AB-PAS (Table 2 and Figure 2).

In addition, 21 out of 26 patients were *H. pylori* (+). The correlation coefficient between *H. pylori* infection and IM (AB-PAS) was 0.41 ($P < 0.05$) (Table 3 and Figure 3).

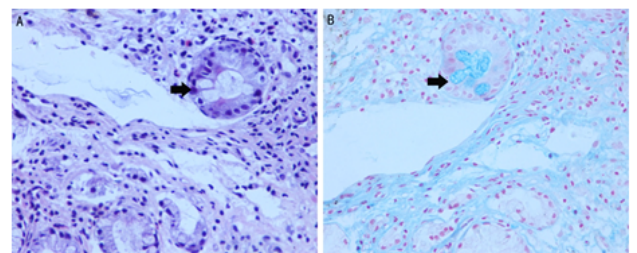


Figure 2. Comparison between HE staining and AB-PAS. A: HE staining of intestinal metaplasia, but there were barely no intestinal metaplasia in that area; B: PAS staining of the same area, where had prominent intestinal metaplasia.

Table 1. Characteristics of enrolled patients.

Characteristics	No
Gender	
Male	22
Female	4
Age (years)	

≤ 60	14
>60	12
Pathological types of tumor	
Adenocarcinoma	24
Gastric stump carcinoma	2
Location of tumor	
Fundus and Body	5
Gastric remnant	2
Antrum	19

Table 2. IM status detected by different staining.

	Total	Positive	Negative	P
HE	52	34	18	0.005
AB	52	43	9	

Table 3. Correlation between IM (AB-PAS) and *H. pylori* infection.

	Total	(+)	(-)	Correlation coefficient	P
IM	26	25	1	0.41	0.038
<i>H. pylori</i>	26	21	5		

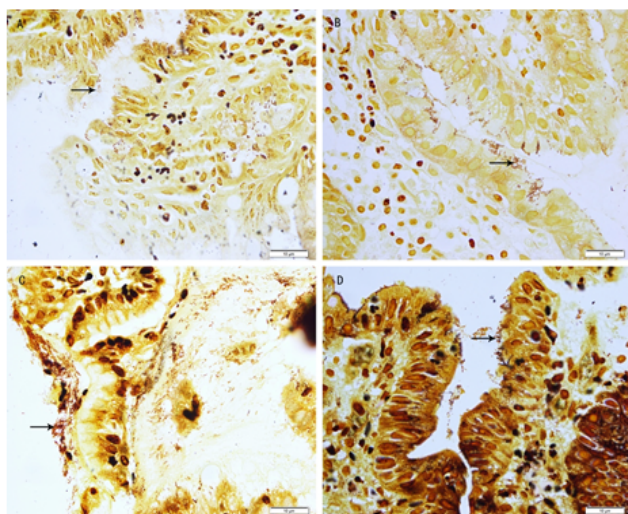


Figure 3. *H. pylori* infection (+), A, B, C and D were Warthin-Starry silver staining of *H. pylori* infected gastric tissues.

Discussion

HE staining is the most common used method in the diagnosis of IM. Although the classifications of IM according to HE staining are comprehensive, it is still complex in procedures and evaluation criteria. IM classifications by AB-PAS are concise and explicit (none, existence). In this study, IM (+) slices in HE staining were also IM (+) in AB-PAS, but a half of IM (-) slices in HE staining were IM (+) in AB-PAS, the

severity of which were all mild. This means AB-PAS may identify earlier IM, but the specific reasons are unknown. It is possible that goblet cells in the gastric mucosa secrete acidic proteins before they become mature. Thus, further studies are needed to confirm the results.

H. pylori are involved in the pathogenesis of GC [9]. Some studies have reported that they contribute to GC by mainly regulating genes (*ras*, *p53*, *c-myc*, etc), cytokines, proliferation and apoptosis of gastric epithelial cells [9,17,18]. GC secondary to IM is also considered as one of consequences due to *H. pylori* infection, but evidence is still conflicting [16]. In this study, the correlation coefficient between *H. pylori* infection and IM was 0.41 (P<0.05), implying a correlation between them. There might be several reasons accountable for this correlation. First, the sample size in this study was small, and only 21 out of enrolled patients were *H. pylori* (+). Second, *H. pylori* can be classified into two types: CagA positive and CagA negative. CagA positive *H. pylori* are highly pathogenic while CagA negative *H. pylori* are not [19]. We did not detect whether *H. pylori* detected in 21 *H. pylori* (+) patients were pathogenic *H. pylori*. Although the correlation coefficient was only 0.41, we still speculate that IM is one of mechanisms underlying the *H. pylori* induced carcinogenesis according to above factors.

Undoubtedly, it will take a remarkably long time evolving from CAG to GC. In our study, it was about 8 months (data not shown). Possible explanation might be that patients visit hospital only when they have some symptoms such as abdominal distension, abdominal pain, and dyspepsia and they might have suffered from CAG for a long time, or even decades before diagnosis. A majority of subjects died at the time of study and thus we were unable to confirm the exact time when patients got sick. More exactly, the “8 months” were the time interval between the diagnosis of CAG and GC.

In the present study, patients with CAG and GC occurring in the same location were recruited in order to assure that GC evolved from CAG, which reduces the error. Previously, it was difficult for gastroscopists to obtain the gastric mucosa from the same point at different time points. Marking Targeting Biopsy (MTB) is now considered as an accurate assisted biopsy technique during gastroscopy. However, repeated injection of MTB solution is necessary for long-term supervision, and may result in inflammation of the gastric mucosa and finally confuse the histopathological evaluation; despite MTB solution was not harmful. Computer Simulation Making Targeting Biopsy (CSMTB) is a technique which can automatically memorize and identify the original lesion of biopsy without solution injection. Ever since the first use in clinical practice, it has never stopped being improved.

There were still several limitations in this study. First, this study was retrospective, and incomplete patients’ information was inevitable. Second, it was difficult to obtain the gastric tissues due to a long time span. More studies are still required before a more preferable method for the diagnosis of IM is developed.

Conclusions

Taken together, AB-PAS is superior to HE staining in the diagnosis of IM, but its clinical application is needed to be further confirmed in future prospective studies. The correlation coefficient between *H. pylori* infection and IM was 0.41, implying an association between them. Thus, IM may be one of mechanisms underlying the *H. pylori* induced carcinogenesis.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (81372623), Zhejiang Province Key Science and Technology Innovation Team (2013TD13), Zhejiang Provincial Medical and Health Research Plan (201476310).

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*Correspondence to

Jianmin Si
 Department of Gastroenterology
 School of Medicine
 Zhejiang University
 PR China
 Shujie Chen
 Institute of Gastroenterology
 Zhejiang University
 PR China