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EDITORIAL

Endoscopic submucosal dissection for early gastric cancer: It is time to consider the quality of its outcomes

Gwang Ha Kim

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Gwang Ha Kim, Internal Medicine, Pusan National University School of Medicine and Biomedical Research Institute, Pusan National University Hospital, Busan 47241, South Korea

Corresponding author: Gwang Ha Kim, MD, PhD, Professor, Internal Medicine, Pusan National University School of Medicine and Biomedical Research Institute, Pusan National University Hospital, 179 Gudeok-ro, Seo-gu, Busan 47241, South Korea. doc0224@pusan.ac.kr

Abstract

Endoscopic resection, particularly endoscopic submucosal dissection (ESD), is widely used as a standard treatment modality for early gastric cancer (EGC) when the risk of lymph node metastasis is negligible. Compared with surgical gastrectomy, ESD is a minimally invasive procedure with additional advantages, such as preservation of the entire stomach and maintenance of the patient's quality of life. However, not all patients achieve curative resection after ESD of EGC. Several patients require surgical gastrectomy after ESD to achieve a curative treatment. Additional surgery after ESD, owing to non-curative resection, places considerable emotional and financial burdens on patients. Recently, as the number of endoscopists performing ESD has increased, the rate of non-curative resection after ESD has increased correspondingly. In order to decrease the non-curative resection rate, as well as determine the ideal rate of non-curative resection after ESD, it is time to consider quality indicators for the outcomes of ESD for EGC.

Key Words: Early gastric cancer; Endoscopic resection; Quality indicator

Core Tip: Endoscopic resection, particularly endoscopic submucosal dissection (ESD), is widely used as a standard treatment modality for early gastric cancer (EGC) when the risk of lymph node metastasis is negligible. Recently, the policy of "diagnostic ESD" has been commonly implemented, especially when accurate prediction of the depth of EGC invasion before ESD is impossible; however, it is neither ideal nor scientific. Therefore, it is time to consider quality indicators for the outcomes of ESD for EGC.

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INTRODUCTION

Gastric cancer (GC) is the fifth most common malignant tumor and the fourth leading cause of cancer-related deaths worldwide[1]. The diagnosis rate of early GC (EGC) has been increasing owing to the widespread use of endoscopy, especially during health checkups, and the development of advanced endoscopy techniques, such as high-definition endoscopy and virtual chromoendoscopy[2-4]. Endoscopic resection, particularly endoscopic submucosal dissection (ESD), is widely used as a standard modality for the curative treatment of EGC when the risk of lymph node metastasis is negligible[5,6]. Compared with surgical gastrectomy, ESD is a minimally invasive procedure with additional advantages, such as preservation of the entire stomach and maintenance of the patient's quality of life. Curative resection after ESD is confirmed based on the following lesion characteristics: (1) Differentiated-type mucosal cancer without ulceration, irrespective of tumor size; (2) Differentiated-type mucosal cancer measuring ≤ 3 cm with ulceration; (3) Differentiated-type cancer measuring ≤ 3 cm with minimal submucosal invasion (depth of invasion into the submucosa < 500 μm); and (4) Undifferentiated-type mucosal cancer measuring ≤ 2 cm without ulceration[5,7]. Although ESD achieves *en bloc*/R0 resection in > 90% of cases, it is not curative in up to 20% of cases. This may be due to previously unsuspected submucosal invasion or horizontal extension, changes in histopathological type (especially to the undifferentiated-predominant mixed type), or lymphovascular invasion on histopathological examination[8]. Therefore, accurate evaluation of the size, invasion depth, horizontal extent, and histopathological type of EGC is essential to improve patient selection for curative ESD.

Advances in ESD techniques and devices, as well as increased opportunities to learn ESD (*e.g.*, visiting ESD training centers, participating in *ex vivo* ESD courses, or watching online or offline videos), have enabled more endoscopists to safely and completely perform ESD in clinical practice, especially in Asian countries. However, not all patients achieve curative resection after ESD of EGC. The main risk factors for non-curative resection are as follows: Tumor location in the upper body, large tumor size (≥ 2 cm), presence of an ulcer, presence of undifferentiated-type component tumor, submucosal invasion, and an inexperienced endoscopist[9-11]. Several patients require surgical gastrectomy after ESD to achieve a curative treatment. Although ESD has merits (*e.g.*, preservation of the stomach and maintenance of quality of life), additional surgery after ESD, owing to non-curative resection, places considerable emotional and financial burdens on patients. Recently, as the number of endoscopists performing ESD has increased, the rate of non-curative resection after ESD has increased correspondingly. In order to decrease the non-curative resection rate, as well as determine the ideal rate of non-curative resection after ESD, we return to the basics.

TIPS FOR SUCCESSFUL ESD OF EGC

Successful ESD of EGC requires accurate prediction of the invasion depth, horizontal extent, and histopathological type of the tumor[2]. To accurately predict the depth of EGC invasion, the macroscopic morphology of the tumor is first considered. Macroscopic findings such as tumor size > 30 mm, remarkable redness, uneven surface, subepithelial tumor-like margin elevation, and mucosal fold convergence are useful for determining the depth of tumor invasion, with a reported accuracy of 83%-97%[12]. However, endoscopic prediction of the invasion depth is subjective and influenced by the endoscopist's level of experience. Endoscopic ultrasonography (EUS) can also be used for determining the depth of EGC invasion, and its overall accuracy with a high-frequency (20 MHz) catheter probe is 81%[13]. EUS is an operator-dependent procedure. In addition, in a retrospective study comparing the accuracy of EUS and conventional endoscopy (based on macroscopic findings) in determining the depth of EGC invasion, EUS was not superior to conventional endoscopy [14]. An integrated diagnostic strategy combining conventional endoscopy and EUS has shown an accuracy of > 85%[15].

The horizontal extent of EGC is mainly determined using conventional endoscopy; however, making accurate prediction becomes challenging when the height and color of the tumor are similar to those of the surrounding normal mucosa. In this situation, chromoendoscopy with indigo carmine alone or indigo carmine and acetic acid, and magnifying endoscopy with narrow-band imaging (ME-NBI) can increase the accuracy of horizontal extent prediction to 90% approximately[16,17]. However, in undifferentiated-type EGC, predicting the horizontal extent using these modalities is challenging. Therefore, during endoscopic examination, biopsy specimens should be obtained from the surrounding tissues and examined histopathologically prior to ESD[12].

The histopathological type of EGC is usually determined based on the results of endoscopic forceps biopsies. However, because these results often do not correctly reflect the final histopathology, histological discrepancies may occur between endoscopic biopsy and ESD-resected specimens. Although the macroscopic morphology and color of lesions have been shown to help predict the histopathological type of EGC, adequate evidence is lacking. Several studies have reported that microsurface and microvascular patterns on ME-NBI can predict the histopathological type of EGC[2,18]. However, systematic ME classification systems, such as those for colorectal polyps and esophageal lesions, have not yet been developed for EGC.

Table 1 Suggested quality indicators for outcomes of endoscopic submucosal dissection for early gastric cancer

Quality indicator	Performance target
En bloc resection rate	> 95%
Complete resection rate	> 90%
Curative resection rate	> 80%
Adverse events	
Post-ESD bleeding	< 10%
Perforation	< 5%

ESD: Endoscopic submucosal dissection.

Hence, other methods to accurately predict the depth of invasion and horizontal extent of EGC are required. Recent studies have reported the use of artificial intelligence (AI) systems for this purpose. Two recent meta-analyses reported that the pooled sensitivity and specificity of AI for predicting deep submucosal invasion were 72%-82% and 79%-90%, respectively[19,20]. In the future, endoscopist-AI cooperation can improve the predictive rates of the depth of invasion, horizontal extent, and histopathological type of EGC before ESD.

CONCLUSION

Recently, the policy of "diagnostic ESD" has been commonly implemented, especially when accurate prediction of the depth of EGC invasion before ESD is impossible. Many young endoscopists have adopted this approach. However, despite its value as diagnostic ESD, it is neither ideal nor scientific. The standard endoscopic process for EGC consists of the following steps: Presence diagnosis (determination of the presence or absence of cancer), qualitative diagnosis (determination of histopathological type), and quantitative diagnosis (determination of depth of invasion and horizontal extent) [2]. Although this process is not perfect, it is recommended for all endoscopists as a basis for every effort to avoid non-curative resection after ESD. Performing ESD without such efforts is likely to increase the non-curative resection rate after ESD for EGC, leading to emotional and economic burdens on patients. Based on the accuracy of endoscopic prediction of the depth of tumor invasion (about 80%) and horizontal tumor extent (about 90%), the ideal non-curative resection rate after ESD is < 15%-20%. According to a recent meta-analysis, *en bloc*, complete, and curative resection rates in Eastern studies were 95% [95% confidence interval (CI): 94%-96%], 89% (95%CI: 88%-91%), and 82% (95%CI: 81%-84%), respectively[21]. EGC cases in which ESD is performed beyond the current ESD indications, considering patient factors such as comorbidities, life expectancy, and the ability to tolerate surgery, should be excluded from this calculation. Furthermore, the main adverse events of ESD, such as post-ESD bleeding and perforation, should be considered when evaluating the quality of ESD outcomes. The overall rates of delayed bleeding and perforation are reported to be 2.6%-8.5% and 2.3%-3.9%, respectively[22-24]. Based on previous reports, I suggest quality indicators for the outcomes of ESD for EGC in Table 1.

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Development of a machine learning-based model for predicting risk of early postoperative recurrence of hepatocellular carcinoma

Yu-Bo Zhang, Gang Yang, Yang Bu, Peng Lei, Wei Zhang, Dan-Yang Zhang

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Yu-Bo Zhang, Gang Yang, Peng Lei, Wei Zhang, Dan-Yang Zhang, Department of Hepatobiliary Surgery, General Hospital of Ningxia Medical University, Yinchuan 750003, Ningxia Hui Autonomous Region, China

Yang Bu, Department of Hepatobiliary Surgery, People's Hospital of Ningxia Hui Autonomous Region, Yinchuan 750003, Ningxia Hui Autonomous Region, China

Corresponding author: Peng Lei, MD, Doctor, Department of Hepatobiliary Surgery, General Hospital of Ningxia Medical University, No. 84 Shenli Nan Road, Yinchuan 750003, Ningxia Hui Autonomous Region, China. leipengnx@126.com

Abstract

BACKGROUND

Surgical resection is the primary treatment for hepatocellular carcinoma (HCC). However, studies indicate that nearly 70% of patients experience HCC recurrence within five years following hepatectomy. The earlier the recurrence, the worse the prognosis. Current studies on postoperative recurrence primarily rely on postoperative pathology and patient clinical data, which are lagging. Hence, developing a new pre-operative prediction model for postoperative recurrence is crucial for guiding individualized treatment of HCC patients and enhancing their prognosis.

AIM

To identify key variables in pre-operative clinical and imaging data using machine learning algorithms to construct multiple risk prediction models for early postoperative recurrence of HCC.

METHODS

The demographic and clinical data of 371 HCC patients were collected for this retrospective study. These data were randomly divided into training and test sets at a ratio of 8:2. The training set was analyzed, and key feature variables with predictive value for early HCC recurrence were selected to construct six different machine learning prediction models. Each model was evaluated, and the best-performing model was selected for interpreting the importance of each variable. Finally, an online calculator based on the model was generated for daily clinical practice.

RESULTS

Following machine learning analysis, eight key feature variables (age, intratumoral arteries, alpha-fetoprotein, pre-operative blood glucose, number of tumors, glucose-to-lymphocyte ratio, liver cirrhosis, and pre-operative platelets) were selected to construct six different prediction models. The XGBoost model outperformed other models, with the area under the receiver operating characteristic curve in the training, validation, and test datasets being 0.993 (95% confidence interval: 0.982-1.000), 0.734 (0.601-0.867), and 0.706 (0.585-0.827), respectively. Calibration curve and decision curve analysis indicated that the XGBoost model also had good predictive performance and clinical application value.

CONCLUSION

The XGBoost model exhibits superior performance and is a reliable tool for predicting early postoperative HCC recurrence. This model may guide surgical strategies and postoperative individualized medicine.

Key Words: Machine learning; Hepatocellular carcinoma; Early recurrence; Risk prediction models; Imaging features; Clinical features

Core Tip: The current study aimed at employing machine learning techniques to select imaging and pre-operative clinical characteristic variables, to which the clinicians were easily accessible, to develop six different risk prediction models for early postoperative recurrence of hepatocellular carcinoma (HCC). We compared the sensitivity and specificity of these models in detecting patients at high risk of early postoperative recurrence of HCC. In addition, to increase the feasibility and applicability of the constructed model, we generated a calculator online based on the predictive model to help clinicians apply it in their daily medical practice.

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INTRODUCTION

Liver cancer is one of the most common malignant tumors globally, and its incidence ranks 5th in various malignant tumors. According to statistics, there were about 905700 new cases of liver cancer worldwide in 2020, and about 830200 patients with liver cancer died. The incidence and mortality of liver cancer are expected to increase by > 50% in 2040[1]. Hepatocellular carcinoma (HCC) is the primary type of liver cancer. Currently, surgical resection is still the main treatment for HCC. However, nearly 70% of patients experience HCC recurrence within five years after hepatectomy[2,3], which is the main factor affecting patients' survival rate after surgery. HCC recurrence can be classified into early (≤ 2 years) or late recurrence (> 2 years after HCC hepatectomy). A study has shown that the earlier the recurrence of HCC, the worse the prognosis, and the overall survival rate of patients with early recurrence of HCC tends to be lower than those with late recurrence[4]. For patients with recurrent HCC, radical treatment is more effective than palliative therapies in prolonging their survival[5,6]. For patients at high risk of potentially postoperative recurrence, neoadjuvant therapy, appropriate intraoperative margin expansion, and early postoperative use of targeted therapies and immuno-therapies may improve their prognosis[7]. However, the lack of specific tumor biomarkers and clinical features makes it challenging to identify the patients at high risk of postoperative recurrence before surgery. Therefore, developing pre-operative non-invasive predictive methods will be highly significant in identifying patients at high risk of postoperative recurrence and precise management of those patients by closely monitoring and individualized treatment on time.

Currently, there are many prediction models to predict the early recurrence of HCC in the clinic[8-10]. However, these models are usually constructed based on linear assumptions, which may not model the complex, multidimensional, and nonlinear relationships among different predictor variables that may exist. Their predictive value is greatly limited[11,12]. In recent years, machine learning has great potential for research in the field of disease prediction because machine learning can better capture the relationships between nonlinearities, deeply mine the hidden relationships in massive data through various algorithms, and build treatment models. These models can efficiently and accurately predict the prognosis of diseases and guide clinical treatment. Previous studies have used artificial intelligence techniques and machine learning algorithms to extract imaging features from pre-operative computed tomography (CT) or magnetic resonance imaging (MRI) images and clinical data to predict the risk of liver cancer recurrence[13,14]. Although these studies make superior predictions, these constructed models remain not highly interpretable and could not be widely applied in most medical scenarios, a common problem with machine learning research.

This study aimed to utilize machine learning techniques to select easily accessible imaging and pre-operative clinical characteristic variables. The goal was to develop six different risk prediction models for the early postoperative re-currence of HCC. We compared the sensitivity and specificity of these models in identifying patients at high risk of early

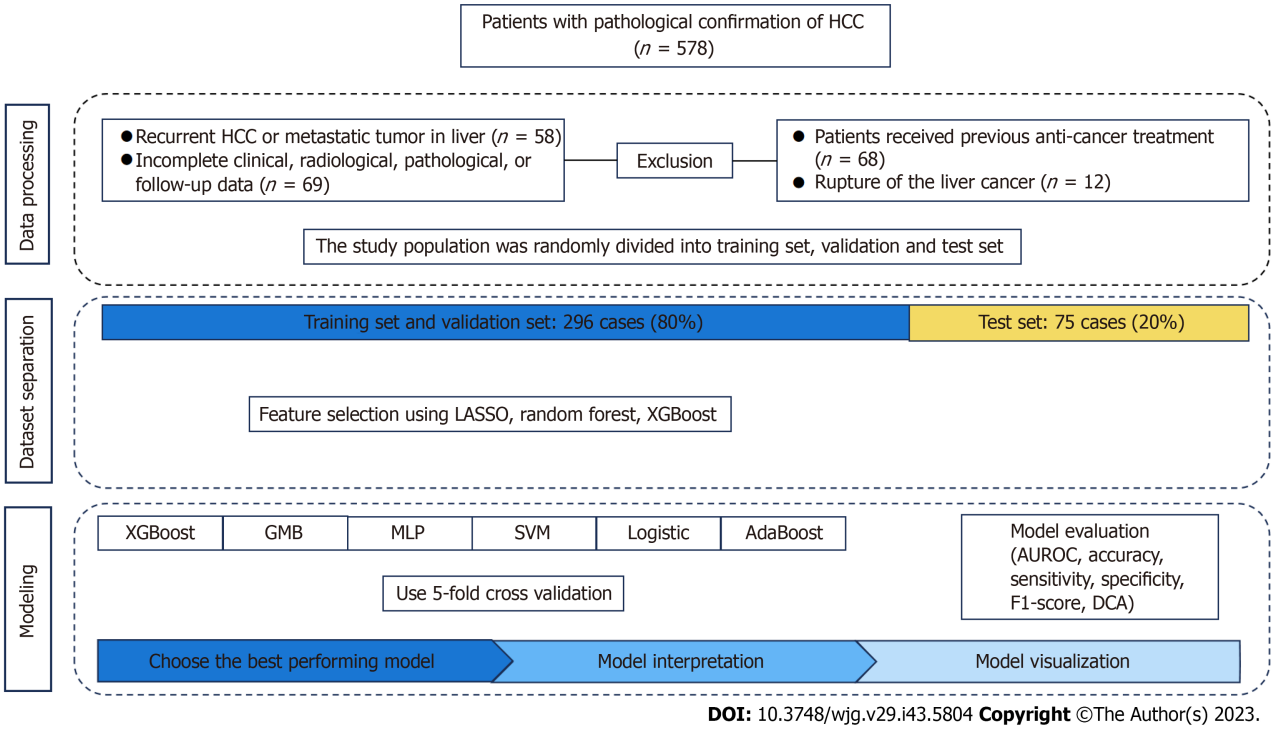

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Figure 1 Flow chart of the study process. HCC: Hepatocellular carcinoma; AUROC: Area under the receiver operating characteristic curve; DCA: Decision curve analysis; GNB: Complement NB; MLP: Multilayer perceptron; SVM: Support vector machine.

postoperative HCC recurrence. Furthermore, to enhance the feasibility and applicability of the constructed model, we created an online calculator based on the predictive model. This tool was designed to assist clinicians in their daily medical practice.

MATERIALS AND METHODS

Research subjects

The demographic and clinical data of age, gender, pathology, radiological images, hepatectomy, and postoperative follow-up of 578 patients with primary liver cancer at Ningxia Medical University General Hospital from January 2017 to June 2021 were retrospectively collected according to the inclusion and exclusion criteria. After excluding those with non-primary liver cancer, incomplete data, receiving neoadjuvant therapy, or liver cancer rupture, the remaining cases were selected for this study, randomized by a simple number generated with a computer, and divided into training and test sets at a ratio of 8:2. The training set of data was used to construct the machine learning model, and the test set of data was used to validate the model performance. The study was approved by the Ethics Committee of the General Hospital of Ningxia Medical University and individual patients provided written informed consent. The study was performed per the Declaration of Helsinki, and the study design followed the TRIPOD statement[15] (Multifactorial predictive reporting norms for individual prognosis or diagnosis). The process of this study is shown in Figure 1.

The inclusion criteria included: (1) Postoperatively pathologically confirmed primary HCC (the first treatment option was radical hepatectomy); (2) undergoing pre-operative abdominal contrast-enhanced MRI examinations; and (3) complete clinical, pathological, and imaging data and postoperative follow-up. The exclusion criteria were: (1) Postoperative pathological diagnosis of secondary HCC; (2) patients undergoing emergency surgery for the ruptured bleeding of liver cancer; (3) receiving pre-operative anti-tumor therapy, such as interventional, ablative, immunologic, or targeted therapy; and (4) acute or chronic infection within 2 wk prior to surgery.

The early liver cancer recurrence was defined according to a previous report[16]. Briefly, a new tumor appeared in the liver within two years after surgical resection of the primary liver cancer. The new tumors were evaluated by radiological imaging or pathological examination. The time to recurrence was defined as the period from the surgical resection of primary liver cancer to the appearance of a new tumor in the liver.

Patients were followed postoperatively in our outpatient service from monthly to every 3 mo in the first-year post-surgical resection of the primary liver cancer. The patients were tested for serum liver functions and alpha-fetoprotein (AFP) levels, chest CT scanning, and abdominal contrast enhanced CT or MRI. Subsequently, the patients who survived were followed every 6 mo starting from the second year after surgery. This follow-up continued until April 2023 or until tumor recurrence, loss of patient follow-up, or death.

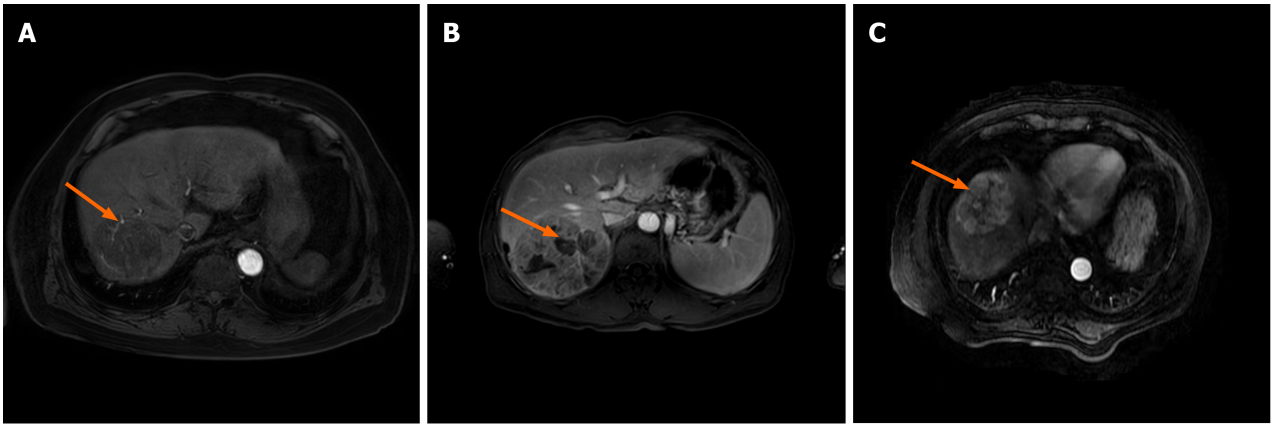

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Figure 2 Representative magnetic resonance imaging images. A: Intratumoral arteries; B: Intratumoral necrosis; C: Uniform tumor enhancement.

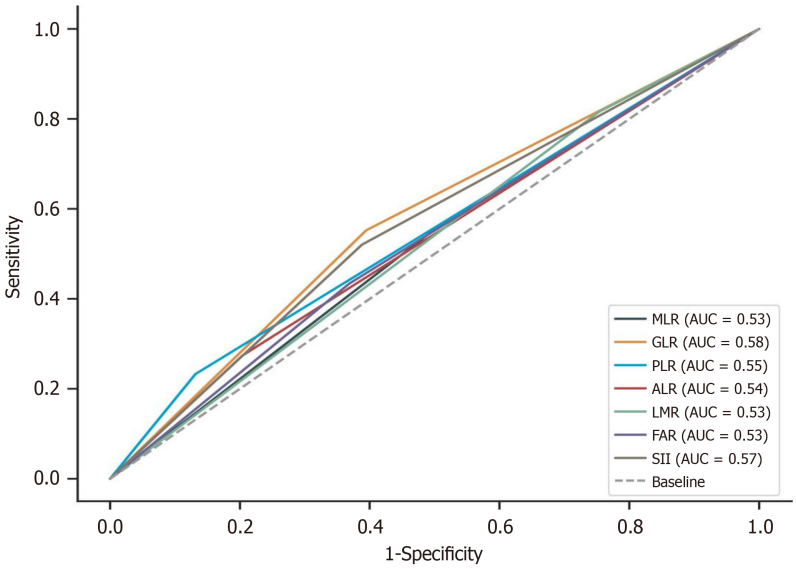

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Figure 3 Receiver operating characteristic curve analyses of diagnostic performance of monocyte to lymphocyte ratio, γ-glutamyl transferase to lymphocyte ratio, platelet count to lymphocyte count ratio, alkaline phosphatase to lymphocyte count ratio, lymphocyte to monocyte count ratio, fibrinogen to albumin level ratio, or systemic immune-inflammation index. AUC: Area under the curve; MLR: Monocyte to lymphocyte ratio; GLR: γ-glutamyl transferase to lymphocyte ratio; PLR: Platelet count to lymphocyte count ratio; ALR: Alkaline phosphatase to lymphocyte count ratio; LMR: Lymphocyte to monocyte count; FAR: Fibrinogen to albumin level ratio; SII: Systemic immune-inflammation index.

Feature variable filtering

The characteristic variables included: (1) Patient's demographic characteristics and pre-operative laboratory tests, such as gender, age, AFP, blood platelet count (PLT), blood glucose, HBeAg, HBsAg, monocyte to lymphocyte ratio (MLR), γ-glutamyl transferase (GGT) to lymphocyte ratio (GLR), PLT to lymphocyte count ratio (PLR), alkaline phosphatase (ALP) to lymphocyte count ratio (ALR), lymphocyte to monocyte count ratio (LMR), fibrinogen to albumin level ratio (FAR), systemic immune-inflammation index (SII; neutrophil count × PLT/lymphocyte count), ALP, and albumin-bilirubin (ALBI) score; and (2) pre-operative imaging features (abdominal contrast-enhanced MRI): Tumor size, tumor number, homogeneous tumor enhancement, intratumoral necrosis, tumor envelope, whether tumor margin is regular, intratumoral arteries, tumor adjacency to major vessels, ascitic fluid, and liver cirrhosis (Figure 2).

Data pre-processing

The data in the training set were analyzed using the receiver operating characteristic (ROC) curve to determine the best cut-off points for the continuous variable values GLR, PLR, ALR, LMR, FAR, and SII to divide the patients into two groups (as shown in Figure 3), where the best cut-off value was 0.285 [95% confidence interval (95%CI): 0.482-0.601] for MLR; 32.16 (95%CI: 0.526- 0.642) for GLR; 138.18 (95%CI: 0.494-0.61) for PLR; 80.29 (95%CI: 0.465-0.583) for ALR; 5.33 (95%CI: 0.40-0.517) for LMR; 0.07 (95%CI: 0.484-0.6) for FAR; and 312.71 (95%CI: 0.507-0.624) for SII in this population. Z-score normalization of continuous variables was performed. When the proportion of missing values was less than 10%,

the mean value was used for interpolation, and the case was deleted when the proportion of missing values of the variable was greater than 10%.

Statistical analysis

All statistical analyses were performed using the R 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria) and Python language. Categorical variables are expressed as *n* (%) and were analyzed by the chi-square test or Fisher's exact test. Continuous variables are expressed as the mean ± SD or the median and interquartile range and were analyzed by the independent-sample *t*-test or the Mann–Whitney *U* test based on their distribution. A *P* value of < 0.05 was considered statistically significant.

The most significant feature variables were chosen from the intersection of the three methods used for important feature analysis. Six different machine learning models were constructed based on these feature sets. The hyperparameters for each model were determined through a grid search to select the best parameters and perform a five-fold cross-validation on the training data set. Cross-validation provided a more accurate evaluation of model performance through metrics from multiple experiments. The predictive performance of the model was validated using discriminative and calibration methods, and the clinical usefulness of the model was evaluated by clinical decision curve analysis (DCA). After selection, the importance of each characteristic in the best model was interpreted using the SHAP package in Python to provide insight into the relationships among them.

RESULTS

Patient characteristics

According to the inclusion and exclusion criteria, 207 out of 578 cases were excluded, and the remaining 371 patients were finally included for this study. Their demographic and clinical characteristics are shown in Table 1. Among them, 219 (59.02%) patients suffered from early HCC recurrence. Those 371 patients were randomized at a ratio of 8:2 into the training and test sets. As a result, 296 patients were in the training set, including 221 males and 75 females, of whom 172 (58.11%) experienced early HCC recurrence; 75 patients in the test set, including 59 males and 16 females, of whom 47 had early HCC recurrence (62.67%). No statistically significant difference was observed in any measure tested between the training and test groups ($P > 0.05$ for all; Table 1).

Feature filtering

A total of 26 potential characteristic variables were selected for this study. Their importance was analyzed using LASSO regression, XGBoost, and random forest (RF). Ten variables with the highest importance were ranked in descending order. Eight key variables were identified after screening the intersection of the variables using the three models. In no particular order, these variables were age, intratumoral arteries, AFP, blood glucose, number of tumors, GLR, liver cirrhosis, and PLT (Figure 4).

Model construction and performance validation

We eventually constructed six machine learning models, and calculated the area under the ROC curve (AUROC) in both the training set and validation set (Figure 5A and B) and generated the forest plots of the area under the curve (AUC) values of the six predictive models in the training set (Figure 6). The XGBoost analyses indicated larger and better AUROCs in the training, validation, and test sets of 0.993 (95%CI: 0.982-1.000), 0.734 (95%CI: 0.601-0.867), and 0.706 (95%CI: 0.585-0.827), respectively (Figure 7). After the five-fold cross-validation, the AUC had an SD of 0.002 for the XGBoost model, the smallest value among the six models, indicating that the XGBoost model had the most stable performance (Figure 6) and that XGBoost achieved lower and better Brier scores (Figure 5C). The DCA results revealed that the XGBoost model was the best diagnostic tool and had good clinical utility compared to other models (Figure 5D). In comparison with individual models (Tables 2 and 3), although the RF model had better indicators for each evaluation in the training dataset, the XGBoost model was an optimal model after a five-fold cross-validation, considering that the RF model might have overfitting. The XGBoost model achieved an AUC of 0.706 (95%CI: 0.585-0.827), accuracy of 0.64, sensitivity of 0.857, specificity of 0.545, and F1 score of 0.766 in the test dataset. The optimal critical value for the prediction probability of the XGBoost model was 55.0 %, according to the Youden index.

The XGBoost model was further analyzed using the SHAP software package, the results unveiled the importance and impact of each feature in the sample, and the importance from the highest to lowest ranked as blood glucose, PLT, intratumoral arteries, AFP, liver cirrhosis, GLR, age, and number of tumors.

Model demonstrations and applications

Analysis of the XGBoost model using the SHAP package indicated that each feature in the sample had both positive and negative impacts. The relationship between the magnitude of the eigenvalues and the predicted impact is shown in Figure 8. For applying the XGBoost model, the optimal cut-off point for the model's prediction probability was 55%. If the model predicted > 55%, it would indicate that patients undergoing hepatectomy are at higher risk of early postoperative HCC recurrence. In addition, we generated a calculator based on the model online for clinicians to apply this model in their daily practice (available at: http://152.136.184.184:5050/).

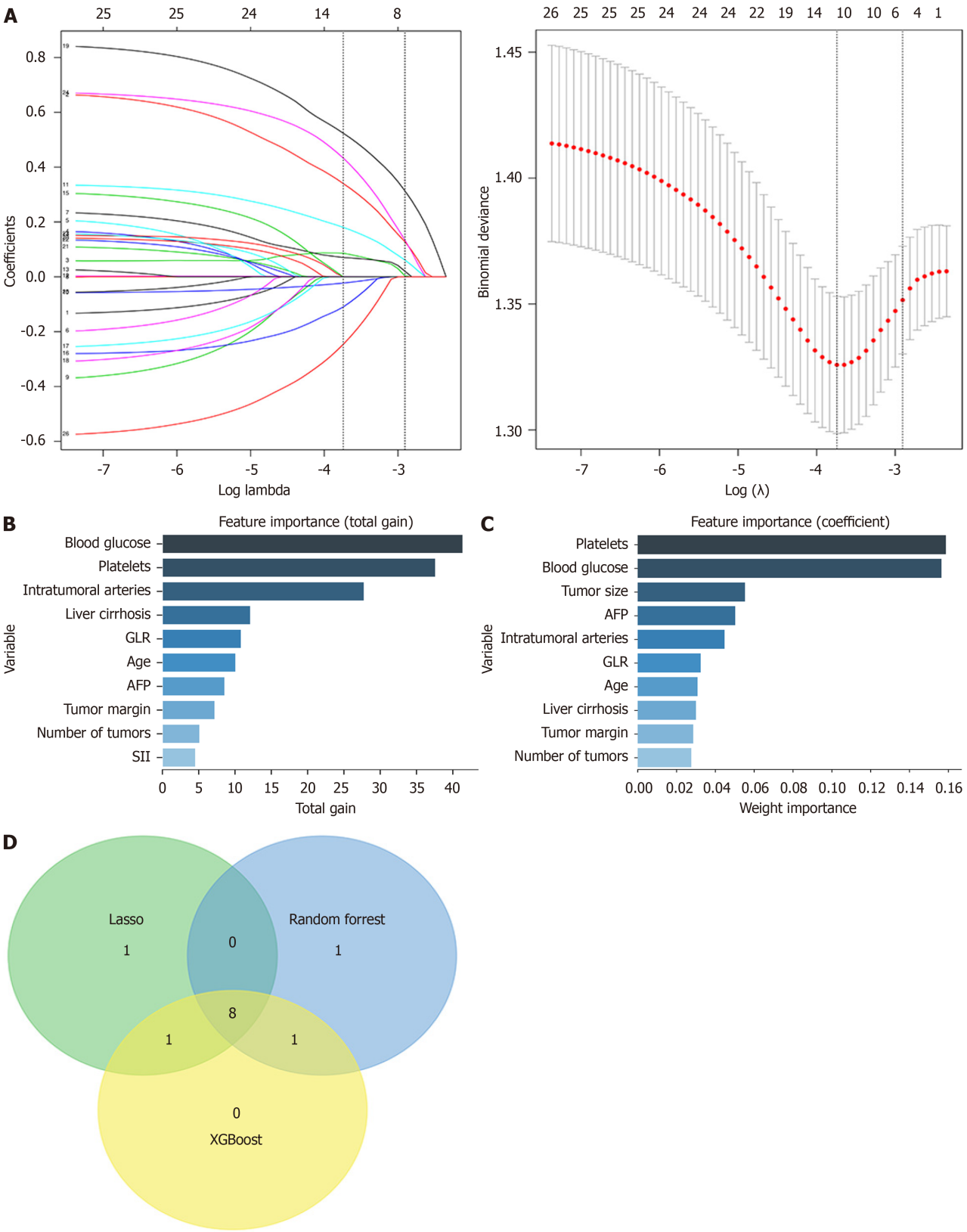

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Figure 4 Importance of feature variables analyzed by LASSO regression, XGBoost, and random forest, and top 10 variables ranked in their importance from the highest to lowest and selected. The Venn diagram was drawn by selecting the features common to all three models (taking the intersection). A: LASSO regression with a vertical line was drawn at the value selected using the ten-fold cross-validation, and a minimum mean square error of λ of 0.024 was chosen to obtain the characteristics of the 11 non-zero coefficients; B: The importance of variable features was analyzed using the XGBoost algorithm; C: The importance of variable features was analyzed using the random forest algorithm; D: Venn diagram with eight key characteristic variables at the intersection: Age, intratumoral arteries, alpha-fetoprotein, blood glucose, number of tumors, γ-glutamyl transferase to lymphocyte ratio, liver cirrhosis, and platelets. AFP: alpha-fetoprotein; GLR: γ-glutamyl transferase to lymphocyte ratio; SII: Systemic immune-inflammation index.

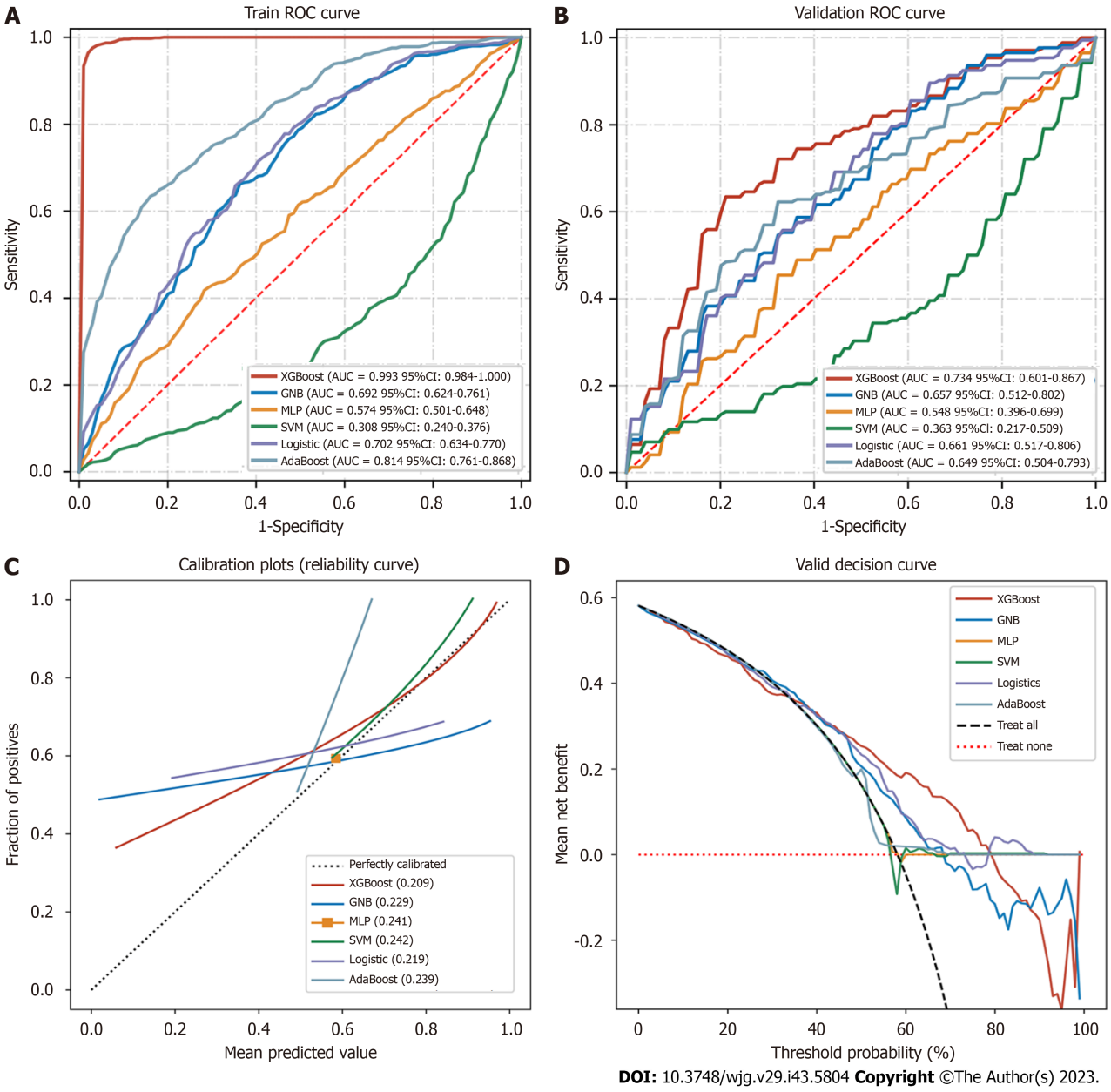

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Figure 5 Receiver operating characteristic analyses and validation of six prediction models. A: Receiver operating characteristic (ROC) curves of the six prediction models in the training dataset; B: ROC curves of the six prediction models in the validation dataset after five-fold cross-validation; C: Calibration plots of nine models. The XGBoost achieved lower (better) Brier scores than the other models; D: Decision curve analysis of six machine learning models. The XGBoost model is the best diagnostic tool for early postoperative hepatocellular carcinoma recurrence. ROC: Receiver operating characteristic.

DISCUSSION

Early HCC recurrence is one of the main factors shortening the survival of postoperative HCC patients. Given that most HCC patients experience HCC recurrence within 5 years post-resection of their primary tumors[2], prevention, early diagnosis, and precise treatment will be critical for limiting postoperative recurrence and improving the survival of HCC patients. This study found an early HCC recurrence rate of 59.02% in this population. While most cases with early HCC recurrence usually come from the hidden micrometastasis of the initial HCC in the body, the late HCC recurrence appears two years after resection. It is commonly from new polyclonal origins of HCC lesions that may completely differ from the initial liver cancer. Actually, early HCC recurrence is usually associated with more aggressive features and a worse prognosis. A previous study has suggested that adjuvant therapy for HCC patients at risk of early recurrence may prolong their survival[17]. However, identifying the patients at high risk of postoperative recurrence before surgery is complicated because of the lack of specific tumor biomarkers and clinical features. Therefore, clinical prediction models will be valuable for predicting early HCC recurrence. However, most available clinical prediction models are often constructed based on the assumption of linear relationships and usually fail to reflect the nature of complex, multidimensional, nonlinear relationships between different predictor variables in the pathogenesis of HCC to affect their predictive performance. Machine learning has shown great potential in analyses of data with nonlinear relationships, and in general, machine learning models outperform traditional regression models in predicting the development of HCC[18].

Table 1 Demographic and clinical characteristics of patients

Variable		Overall (*n* = 371)	No HCC recurrence (*n* = 152)	HCC recurrence (*n* = 219)	*P* value	Training set (*n* = 296)	Test set (*n* = 75)	*P* value
Liver cirrhosis, *n* (%)	No	85 (22.911)	28 (18.421)	57 (26.027)	0.086	85 (22.911)	69 (23.311)	0.716
	Yes	286 (77.089)	124 (81.579)	162 (73.973)		227 (76.689)	59 (78.667)	
Tumor size, *n* (%), cm	≤ 2	42 (11.321)	16 (10.526)	26 (11.872)	0.027	29 (9.797)	13 (17.333)	0.308
	2-5	168 (45.283)	78 (51.316)	90 (41.096)		137 (46.284)	31 (41.333)	
	5-10	109 (29.380)	46 (30.263)	63 (28.767)		87 (29.392)	22 (29.333)	
	≥ 10	52 (14.016)	12 (7.895)	40 (18.265)		43 (14.527)	9 (12.000)	
Tumor number, *n* (%)	1	285 (76.819)	128 (84.211)	157 (71.689)	0.005	229 (77.365)	56 (74.667)	0.621
	≥ 2	86 (23.181)	24 (15.789)	62 (28.311)		67 (22.635)	19 (25.333)	
Homogeneous tumor enhancement, *n* (%)	No	297 (80.054)	119 (78.289)	178 (81.279)	0.479	238 (80.405)	59 (78.667)	0.736
	Yes	74 (19.946)	33 (21.711)	41 (18.721)		58 (19.595)	16 (21.333)	
Intratumoral necrosis, *n* (%)	No	142 (38.275)	69 (45.395)	73 (33.333)	0.019	113 (38.176)	29 (38.667)	0.938
	Yes	229 (61.725)	83 (54.605)	146 (66.667)		183 (61.824)	46 (61.333)	
Tumor envelope intactness, *n* (%)	No	145 (39.084)	60 (39.474)	85 (38.813)	0.898	117 (39.527)	28 (37.333)	0.728
	Yes	226 (60.916)	92 (60.526)	134 (61.187)		179 (60.473)	47 (62.667)	
Regular tumor margin, *n* (%)	No	176 (47.439)	73 (48.026)	103 (47.032)	0.850	139 (46.959)	37 (49.333)	0.713
	Yes	195 (52.561)	79 (51.974)	116 (52.968)		157 (53.041)	38 (50.667)	
Intratumoral arteries, *n* (%)	No	174 (46.900)	89 (58.553)	85 (38.813)	< 0.001	135 (45.608)	39 (52.000)	0.322
	Yes	197 (53.100)	63 (41.447)	134 (61.187)		161 (54.392)	36 (48.000)	
Adjacency to major vessels, *n* (%)	No	216 (58.221)	93 (61.184)	123 (56.164)	0.335	171 (57.770)	45 (60.000)	0.727
	Yes	155 (41.779)	59 (38.816)	96 (43.836)		125 (42.230)	30 (40.000)	
Ascitic fluid, *n* (%)	No	262 (70.620)	106 (69.737)	156 (71.233)	0.756	207 (69.932)	55 (73.333)	0.564
	Yes	109 (29.380)	46 (30.263)	63 (28.767)		89 (30.068)	20 (26.667)	
Age, *n* (%), yr	< 60	227 (61.186)	85 (55.921)	142 (64.840)	0.083	187 (63.176)	40 (53.333)	0.118
	≥ 60	144 (38.814)	67 (44.079)	77 (35.160)		109 (36.824)	35 (46.667)	
Gender, *n* (%)	Male	280 (75.472)	118 (77.632)	162 (73.973)	0.421	221 (74.662)	59 (78.667)	0.472
	Female	91 (24.528)	34 (22.368)	57 (26.027)		75 (25.338)	16 (21.333)	
HBsAg, *n* (%)	Negative	101 (27.224)	41 (26.974)	60 (27.397)	0.928	76 (25.676)	25 (33.333)	0.183
	Positive	270 (72.776)	111 (73.026)	159 (72.603)		220 (74.324)	50 (66.667)	
HBeAg, *n* (%)	Negative	289 (77.898)	123 (80.921)	166 (75.799)	0.242	229 (77.365)	60 (80.000)	0.623
	Positive	82 (22.102)	29 (19.079)	53 (24.201)		67 (22.635)	15 (20.000)	
ALBI, *n* (%), grade	1	67 (18.059)	26 (17.105)	41 (18.721)	0.907	54 (18.243)	13 (17.333)	0.710
	2	288 (77.628)	119 (78.289)	169 (77.169)		228 (77.027)	60 (80.000)	
	3	16 (4.313)	7 (4.605)	9 (4.110)		14 (4.730)	2 (2.667)	
MLR, *n* (%)	< 0.285	171 (46.092)	75 (49.342)	96 (43.836)	0.295	140 (47.297)	31 (41.333)	0.355
	≥ 0.285	200 (53.908)	77 (50.658)	123 (56.164)		156 (52.703)	44 (58.667)	
GLR, *n* (%)	< 32.16	190 (51.213)	92 (60.526)	98 (44.749)	0.003	152 (51.351)	38 (50.667)	0.916

	≥ 32.16	181 (48.787)	60 (39.474)	121 (55.251)		144 (48.649)	37 (49.333)	
PLR, *n* (%)	< 138.18	300 (80.863)	132 (86.842)	168 (76.712)	0.015	241 (81.419)	59 (78.667)	0.588
	≥ 138.18	71 (19.137)	20 (13.158)	51 (23.288)		55 (18.581)	16 (21.333)	
ALP, *n* (%)	< 80.29	280 (75.472)	121 (79.605)	159 (72.603)	0.123	222 (75.000)	58 (77.333)	0.675
	≥ 80.29	91 (24.528)	31 (20.395)	60 (27.397)		74 (25.000)	17 (22.667)	
LMR, *n* (%)	< 5.33	82 (22.102)	28 (18.421)	54 (24.658)	0.155	67 (22.635)	15 (20.000)	0.623
	≥ 5.33	289 (77.898)	124 (81.579)	165 (75.342)		229 (77.365)	60 (80.000)	
FAR, *n* (%)	< 0.07	220 (59.299)	96 (63.158)	124 (56.621)	0.208	174 (58.784)	46 (61.333)	0.688
	≥ 0.07	151 (40.701)	56 (36.842)	95 (43.379)		122 (41.216)	29 (38.667)	
SII, *n* (%)	< 312.71	198 (53.369)	93 (61.184)	105 (47.945)	0.012	157 (53.041)	41 (54.667)	0.801
	≥ 312.71	173 (46.631)	59 (38.816)	114 (52.055)		139 (46.959)	34 (45.333)	
AFP, *n* (%), ng/mL	< 20	171 (46.092)	81 (53.289)	90 (41.096)	0.012	138 (46.622)	33 (44.000)	0.857
	20-400	86 (23.181)	37 (24.342)	49 (22.374)		69 (23.311)	17 (22.667)	
	≥ 400	114 (30.728)	34 (22.368)	80 (36.530)		89 (30.068)	25 (33.333)	
PLT, median (IQR), 109/L		168.000 (113.000, 209.000)	154.000 (112.000, 200.000)	171.000 (118.000, 216.000)	0.110	165.000 (111.000, 208.000)	178.000 (133.000, 220.000)	-1.451
Blood glucose, median (IQR), mmol/L		4.720 (4.340, 5.310)	4.840 (4.390, 5.660)	4.660 (4.300, 5.170)	0.009	4.710 (4.350, 5.310)	4.770 (4.300, 5.310)	-0.066

HCC: Hepatocellular carcinoma; ALBI: Albumin-bilirubin; MLR: Monocyte to lymphocyte ratio; GLR: γ-glutamyl transferase to lymphocyte ratio; PLR: Platelet count to lymphocyte count ratio; ALR: Alkaline phosphatase to lymphocyte count ratio; LMR: Lymphocyte count to monocyte count; FAR: Fibrinogen to albumin ratio; SII: Systemic immune-inflammation index; PLT: Blood platelets; AFP: Alpha-fetoprotein; IQR: Interquartile range.

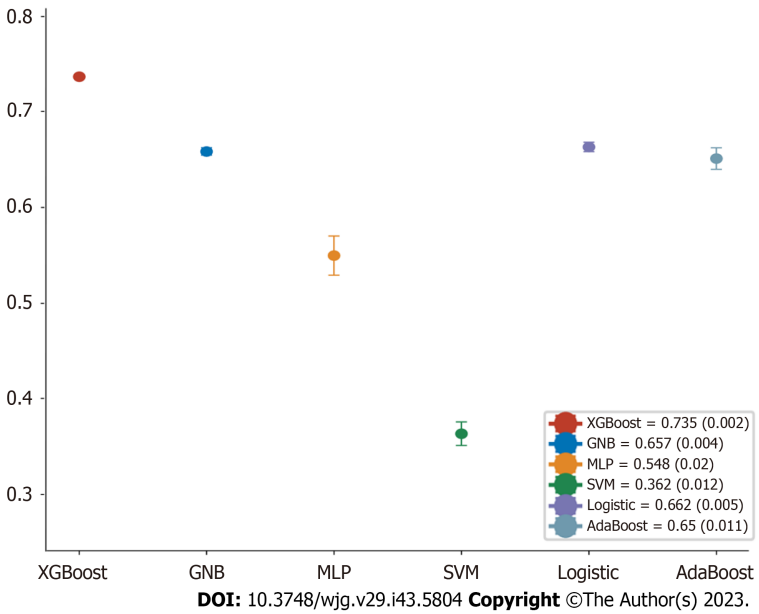

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Figure 6 Forest plot of the area under the curve scores of the six models. The XGBoost model achieved a smaller (better) SD compared with the other models. GNB: Complement NB; MLP: Multilayer perceptron; SVM: Support vector machine.

In this study, we analyzed pre-operative imaging and clinical indicators that were easily accessible and non-invasive and selected eight important feature variables using machine learning algorithms, including pre-operative blood glucose value, PLT, intratumoral artery mass, methemoglobin, cirrhosis, GLR, age, and number of tumors. Subsequently, we generated six machine learning prediction models and validated their value in predicting the risk of early postoperative HCC recurrence. After randomly dividing the independent test set, we applied hyperparameter tuning in the training dataset with five-fold cross-validation and validated them in the test dataset. The results indicated that the XGBoost model exhibited better discriminative and predictive value than other models constructed. The calibration plots for the

Table 2 Performance metrics of six models in the training dataset

Model	AUC (95%CI)	Accuracy (95%CI)	Sensitivity (95%CI)	Specificity (95%CI)	F1 score (95%CI)
XGBoost	0.993 (0.984-1.000)	0.974 (0.969-0.979)	0.980 (0.971-0.988)	0.976 (0.966-0.986)	0.981 (0.977-0.985)
GNB	0.692 (0.624-0.761)	0.671 (0.660-0.683)	0.754 (0.689-0.820)	0.566 (0.486-0.646)	0.728 (0.705-0.750)
MLP	0.574 (0.501-0.648)	0.563 (0.516-0.611)	0.517 (0.274-0.761)	0.637 (0.410-0.863)	0.549 (0.411-0.686)
SVM	0.308 (0.240-0.376)	0.453 (0.391-0.514)	0.204 (-0.182-0.591)	0.800 (0.413-1.187)	NaN
Logistic	0.702 (0.634-0.770)	0.677 (0.657-0.696)	0.747 (0.654-0.841)	0.589 (0.499-0.679)	0.727 (0.689-0.765)
AdaBoost	0.814 (0.761-0.868)	0.725 (0.701-0.748)	0.661 (0.585-0.737)	0.825 (0.773-0.876)	0.737 (0.700-0.773)

95%CI: 95% confidence interval; AUC: Area under the curve; GNB: Complement NB; MLP: Multilayer perceptron; SVM: Support vector machine; NaN: Not a number.

Table 3 Performance metrics of six models in the validation dataset

Model	AUC (95%CI)	Accuracy (95%CI)	Sensitivity (95%CI)	Specificity (95%CI)	F1 score (95%CI)
XGBoost	0.734 (0.601-0.867)	0.683 (0.658-0.708)	0.662 (0.596-0.729)	0.782 (0.717-0.847)	0.694 (0.636-0.752)
GNB	0.657 (0.512-0.802)	0.618 (0.583-0.653)	0.647 (0.443-0.851)	0.662 (0.464-0.861)	0.641 (0.535-0.748)
MLP	0.548 (0.396-0.699)	0.514 (0.452-0.575)	0.575 (0.398-0.752)	0.627 (0.491-0.764)	0.577 (0.471-0.682)
SVM	0.363 (0.217-0.509)	0.446 (0.381-0.511)	0.452 (0.035-0.870)	0.617 (0.188-1.046)	NaN
Logistic	0.661 (0.517-0.806)	0.632 (0.602-0.661)	0.797 (0.709-0.886)	0.525 (0.414-0.637)	0.728 (0.706-0.751)
AdaBoost	0.649 (0.504-0.793)	0.618 (0.569-0.667)	0.591 (0.330-0.852)	0.780 (0.610-0.950)	0.617 (0.403-0.831)

95%CI: 95% confidence interval; AUC: Area under the curve; GNB: Complement NB; MLP: Multilayer perceptron; SVM: Support vector machine; NaN: Not a number.

XGBoost model also displayed an excellent consistency between predictions and actual observations, and the DCA unveiled that the XGBoost model exhibited excellent clinical application. The model was, therefore, selected as the optimal model with an optimal cut-off value of 55% for the prediction probability. If a patient has a predicted probability of greater than 55%, the patient will have an increased risk of early HCC recurrence after primary tumor resection. Therefore, the prediction model may be valuable for predicting HCC patients for their risk of early postoperative HCC recurrence. Accordingly, to make the prediction model more convenient for clinical practice, we generated a model-based calculator online for physicians to use this model to predict early postoperative HCC recurrence. This will significantly improve the efficiency of the practical application of the prediction model.

To ensure better performance and clinical interpretation of the predictive model, we used the shape model to analyze its interpretation after determining the importance of the relationship between each characteristic variable and early postoperative HCC recurrence. The results indicated that the pre-operative blood glucose value was the variable with the highest importance, consistent with previous studies[19-21]. Hyperglycemia has now been recognized as a risk factor for the development and prognosis of HCC. Shi *et al*[19] found that HCC cells employed glycolysis to support their rapid growth in hypoxic and oxidative stress in the body. On the other hand, hyperglycemia can enhance the growth, invasion, and metastasis of HCC cells by promoting the expression of vascular endothelial growth factor[20]. Similarly, PLT also influenced the early HCC recurrence more, possibly by a positive feedback regulation[22]. Elevated pre-operative PLT are independently associated with increased tumor load, extrahepatic recurrence and metastasis, and poor prognoses of HCC [23]. In turn, HCC cells can promote platelet generation by secreting platelet-generating hormones, and the increased PLT further provide favorable conditions for HCC cell proliferation and metastasis[24,25]. Intratumoral arterial features on pre-operative imaging are a significant risk factor for early postoperative HCC recurrence. A previous study has shown that the distribution and number of blood vessels supplying the tumor can impact the prognosis of HCC patients[26]. Multiple small arteries supplying blood to the tumor may reflect high pressure inside the tumor. The high-pressure environment tends to cause the tumor cells to shed and form microvascular invasion in the surrounding vessels, which may even spread to other locations in the liver, increasing the risk of early postoperative HCC recurrence. Moreover, inflammatory factors are essential for the tumor microenvironment, and high levels of GGT will lead to high cysteine levels *in vivo*, which influence tumor development regarding redox regulation and drug resistance[27]. GGT is a new potential prognostic indicator of inflammation in HCC[28], while lymphocytes are crucial for the systemic and local immune responses, including anti-tumor immunity[29]. A decrease in the number of lymphocytes can result in a weakened immune function, which may enhance angiogenesis and extracellular matrix remodeling, creating a mutagenic environment for tumor cells and promoting tumorigenesis and development[30]. Indeed, GLR has been shown to be

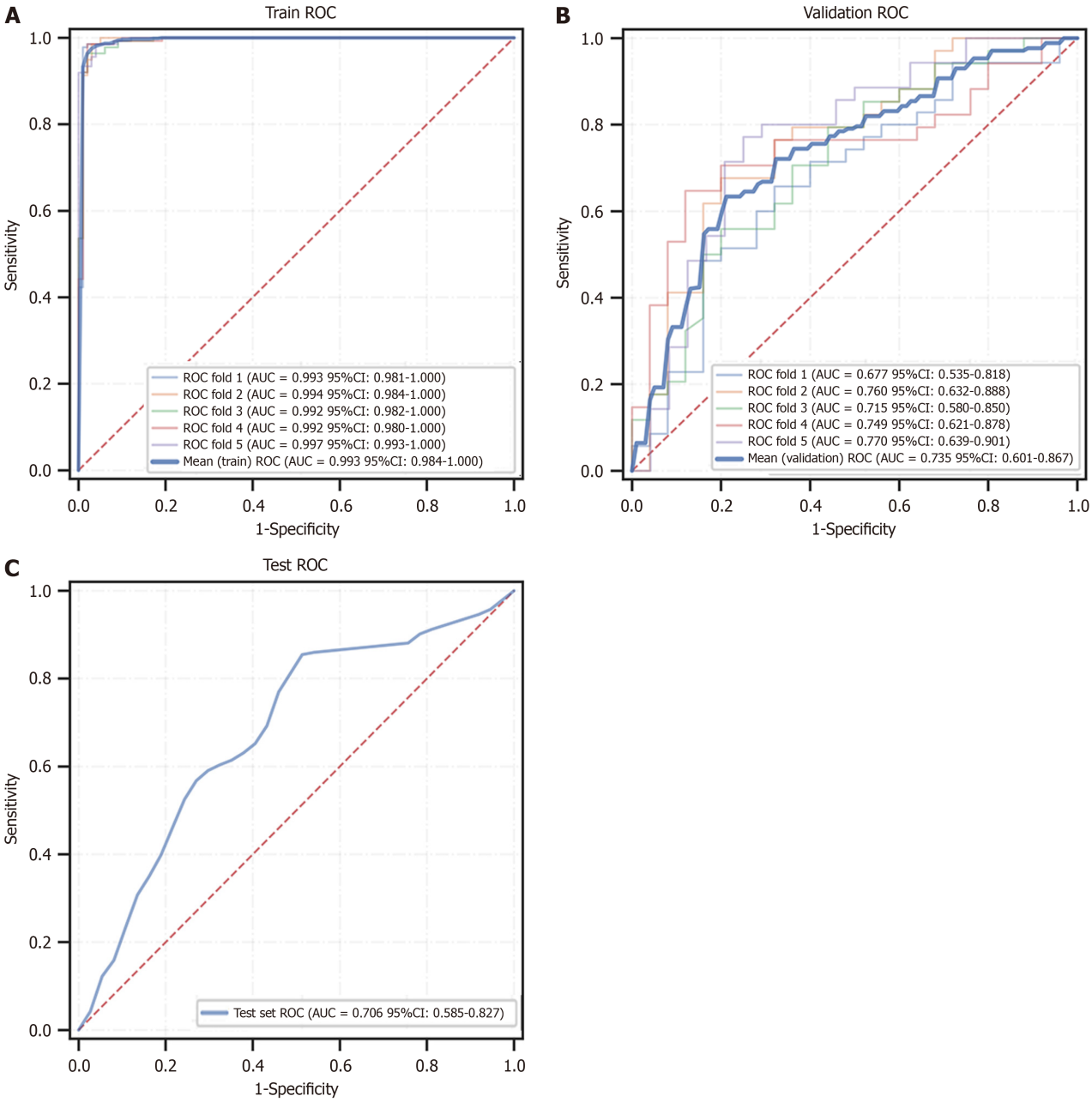

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Figure 7 Receiver operating characteristic curves of the XGBoost model in training, validation, and test datasets. ROC: Receiver operating characteristic; 95%CI: 95% confidence interval.

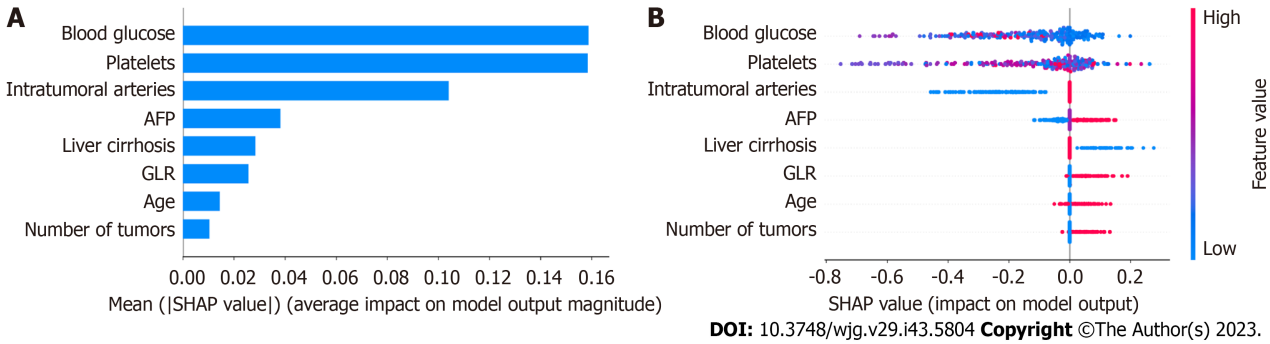
valuable for predicting early postoperative HCC recurrence in a population of 606 patients[31]. Therefore, these vital feature variables included in the best mode are critical for predicting early postoperative HCC recurrence.

In summary, the predictive model constructed in this study may help clinicians predict the risk of early postoperative HCC recurrence in individual HCC patients and better manage them before, during, and after surgery. Our findings may open new avenues for investigating therapeutic strategies for postoperative recurrence of HCC.

We acknowledge that the current study has limitations. First, our study was conducted with a relatively small sample size in a single center and did not include external validation from other centers. Therefore, future studies are needed to validate the value of this model in a larger patient population across multiple centers. Second, the optimal thresholds for the inflammation-related ratio in this study may need to be re-evaluated due to the limited sample size. Considering these limitations, we plan to expand the sample size through a multicenter study in the future and compare our model with other prediction models to further verify its reliability.

CONCLUSION

Compared to other models, the XGBoost model demonstrated superior performance and emerged as the best predictive



DOI: 10.3748/wjg.v29.i43.5804

Figure 8 SHAP analysis of the XGBoost model. A: Visual representation of each feature in the XGBoost model and the relationship between the importance of each feature. The color represents the value of the variable, with red representing the larger value and blue representing the smaller value. AFP: Alpha-fetoprotein; GLR: γ-glutamyl transferase to lymphocyte ratio.

measurement model. This predictive model is easily accessible in daily clinical practice and may serve as a crucial tool in guiding postoperative follow-up and individualized medicine for HCC patients.

ARTICLE HIGHLIGHTS

Research background

Surgical resection is still the main treatment for hepatocellular carcinoma (HCC). HCC recurrence is the main factor affecting patients' survival rate after surgery. Developing pre-operative non-invasive predictive methods will be highly significant in identifying patients at high risk of postoperative recurrence and precise management of those patients by closely monitoring and individualized treatment on time.

Research motivation

To develop a new risk prediction model for the early postoperative recurrence of HCC and enhance the feasibility and applicability of the constructed model.

Research objectives

This study aimed to identify key variables in pre-operative clinical and imaging data using machine learning algorithms to construct multiple risk prediction models for early postoperative recurrence of HCC.

Research methods

The demographic and clinical data of 371 HCC patients were collected and analyzed, and the key feature variables were selected to construct six different machine learning prediction models. Each model was evaluated, and the best-performing model was selected for interpreting the importance of each variable. Finally, an online calculator based on the model was generated for daily clinical practice.

Research results

Following machine learning analysis, eight key feature variables were selected to construct six different prediction models. The XGBoost model outperformed other models, with the area under the receiver operating characteristic curve in the training, validation, and test datasets being 0.993 [95% confidence interval (95%CI): 0.982-1.000], 0.734 (95%CI: 0.601-0.867), and 0.706 (95%CI: 0.585-0.827), respectively. Calibration curve and decision curve analysis indicated that the XGBoost model also had good predictive performance and clinical application value.

Research conclusions

The XGBoost model exhibits superior performance and is a reliable tool for predicting early postoperative HCC recurrence. This model may guide surgical strategies and postoperative individualized medicine.

Research perspectives

A multicenter study with large samples should be conducted in the future, and comparing our model with other prediction models is needed to further verify its reliability.

FOOTNOTES

Author contributions: Zhang YB analyzed the data and wrote the manuscript; Yang G analyzed the data and wrote the original draft; Bu

Y contributed the resources; Lei P designed the research; Zhang W analyzed the data; Zhang DY wrote the original draft.

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ORCID number: Yu-Bo Zhang 0009-0005-6486-8161; Gang Yang 0009-0004-3262-0770; Yang Bu 0000-0003-1219-997X; Peng Lei 0000-0002-5225-485X; Wei Zhang 0009-0004-4607-8396; Dan-Yang Zhang 0009-0002-3606-6020.

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ORIGINAL ARTICLE

Observational Study

Knowledge, attitude, and practice of patients living with inflammatory bowel disease: A cross-sectional study

Xiao-Xiao Shao, Lu-Yan Fang, Xu-Ri Guo, Wei-Zhong Wang, Rui-Xin Shi, Dao-Po Lin

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Xiao-Xiao Shao, Lu-Yan Fang, Xu-Ri Guo, Dao-Po Lin, Department of Gastroenterology, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou 325027, Zhejiang Province, China

Wei-Zhong Wang, Rui-Xin Shi, The Second Clinical Medical College, Wenzhou Medical University, Wenzhou 325035, Zhejiang Province, China

Corresponding author: Dao-Po Lin, MD, Department of Gastroenterology, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, No. 109 Xueyuan Western Road, Wenzhou 325027, Zhejiang Province, China. wmuldp@163.com

Abstract

BACKGROUND

Patients with inflammatory bowel diseases (IBDs) generally have poor knowledge, attitude, and practice of their disease, while the data from China are lacking.

AIM

To address this knowledge disparity among Chinese patients with IBD.

METHODS

This web-based, cross-sectional study was conducted on a cohort of IBD patients who visited the Second Affiliated Hospital of Wenzhou Medical University between December 2022 and February 2023. Their socio-demographic information and the knowledge, attitude, and practice scores were collected and estimated using a self-designed questionnaire. Pearson's correlation analysis was used to determine the pairwise correlations among knowledge, attitude, and practice scores. A multivariate logistic regression analysis was further performed to determine the independent factors associated with their knowledge, attitude, and practice scores.

RESULTS

A total of 353 patients (224 males) with IBD completed the questionnaires. The mean knowledge, attitude, and practice scores were 10.05 ± 3.46 (possible range: 0-14), 41.58 ± 5.23 (possible range: 0-56), 44.20 ± 7.39 (possible range: 0-56), respectively, indicating good knowledge, positive attitude, and proactive practice toward IBD. Pearson's correlation analysis showed that the knowledge score had significant positive correlations with the attitude score (r = 0.371, P < 0.001) and

practice score (r = 0.100, P < 0.001). The attitude score had a significant positive correlation with the practice score (r = 0.452, P < 0.001). Moreover, multivariate logistic regression analysis showed that aged 30-40 years [odds ratio (OR) = 4.06, 95% confidence interval (CI): 1.04-15.82, P = 0.043], middle school education (OR = 3.98, 95%CI: 1.29-12.33, P = 0.017), high school/technical secondary school education (OR = 14.06, 95%CI: 3.92-50.38, P < 0.001), and junior college/bachelor's degree and above education (OR = 15.20, 95%CI: 4.15-55.650, P < 0.001) were independently associated with good knowledge. The higher knowledge score was independently associated with a positive attitude (OR = 1.23, 95%CI: 1.11-1.36, P < 0.001). The higher attitude score was independently associated with proactive practice (OR = 1.20, 95%CI: 1.11-1.30, P < 0.001).

CONCLUSION

Chinese patients with IBD might have good knowledge, a positive attitude, and proactive practice toward their disease. However, a small number of specific items require education.

Key Words: Attitude; Cross-sectional study; Inflammatory bowel disease; Knowledge; Practice; Questionnaire

Core Tip: To address this knowledge disparity among Chinese patients with inflammatory bowel disease (IBD), a web-based, cross-sectional study was conducted on 353 IBD patients (224 males). Their mean knowledge, attitude, and practice scores were 10.05 ± 3.46 (range: 0-14), 41.58 ± 5.23 (range: 0-56), and 44.20 ± 7.39 (range: 0-56), respectively. Multivariate logistic regression analysis showed that age and education were independently associated with knowledge. Knowledge was independently associated with attitude. The attitude was independently associated with the practice. In conclusion, patients with IBD in China might have good knowledge, a positive attitude, and proactive practice toward their disease. However, some specific items require education.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic, non-specific inflammation of the gastrointestinal tract, including ulcerative colitis and Crohn's disease. The IBD usually develops before age 30[1-3]. Moreover, IBD is associated with a poor quality of life and may increase colorectal cancer risk[2-4]. The individual management strategy of IBD is tailored to each patient according to diagnosis, disease activity grade, disease lesion, and personal prognostic factors[1,3-7]. Despite this, IBD continues to be difficult to manage, as treatment adverse effects and repeated exacerbation/recurrence episodes can eventually necessitate costly second-line therapies or even surgery[8-11].

Maintaining proper lifestyle habits is necessary and complementary to medical treatments in patients with IBD[12,13]. Fundamental to patient self-management is knowing which foods and situations to avoid and what can be done to alleviate symptoms[12,13]. To implement adequate self-management, a thorough understanding of IBD causes, risk factors for exacerbation/recurrence, disease mechanisms, and treatments is essential, and this knowledge needs to be translated into more effective (but not infallible) self-management. In addition, since there is no cure for IBD, self-management is essential to its treatment[1,5,14]. Indeed, since the management of IBD necessitates the adoption of healthy lifestyle habits, IBD patients are the first to be accountable for their health[12,13], which requires proper knowledge, attitudes, and practice (KAP) of the specific lifestyle routines to implement. The appropriate KAP about IBD can reduce medical acceleration in patients with IBD[15]. Since the 1990s, however, some studies have revealed that patients with IBD have misconceptions and limited knowledge of their disease[16-21]. Such studies are important to identify the gaps in knowledge that represent barriers to the proper management of IBD. Identifying these obstacles could also aid in designing interventions to enhance or rectify knowledge[22,23]. Owing to significant differences in culture, economy, health literacy, healthcare systems, and government policies, KAP data are usually very specific to a given population. Of note, data about the KAP toward IBD in Chinese patients with IBD are lacking.

The KAP methodology provides quantitative and qualitative data on the misconceptions that could represent obstacles to a specific task/subject in a specific population[22,23]. Hence, this study aimed to investigate the KAP of patients with IBD toward their disease in Zhejiang Province, China. The results could help healthcare providers to improve the patient's self-management of IBD.

MATERIALS AND METHODS

Study design and participants

It was a cross-sectional study conducted on patients with IBD at the Second Affiliated Hospital of Wenzhou Medical University using convenience sampling. Our study was approved by the ethics committee of the same hospital (approval No. 2022-K-184-02). Each patient provided written informed consent before completing the survey.

Procedures

The questionnaire was designed with reference to the World Gastroenterology Organization Practice Guidelines for the Diagnosis and Management of IBD in 2010[24] and the clinical nutrition guideline for IBD by the European Society for Clinical Nutrition and Metabolism in 2016[25]. Then, the questionnaire was submitted to 5 experts for review. After the modifications based on their comments, a small-scale validation was performed (33 copies), showing a Cronbach's α of 0.854.

The final questionnaire was in Chinese patients with IBD and included four dimensions with 62 items. Among them, the socio-demographic information dimension consisted of 20 items. The knowledge, attitude, and practice dimensions consisted of 14 items each. The items in the knowledge dimension were scored 1 point for a correct answer and 0 points for a wrong or unclear answer (total score of 0-14). The options from positive to negative (*e.g.*, 4 to 0) were assigned for the attitude and practice dimension. The total scores were 0-56 for the attitude dimension and 0-56 for the practice dimension. The threshold for good knowledge, positive attitude, and proactive practice was ≥ 70.0%.

The questionnaires were administered to the participants through WeChat on the SoJump platform (https://www.wjx.cn/app/survey.aspx). A given IP address could be used to submit a questionnaire only once. All items must be answered before the submission of the questionnaire. Questionnaires that took less than 2 min to complete or with obvious filling patterns were excluded.

Statistics analysis

All analyses were performed using Stata 17.0 (Stata Corporation, College Station, TX, United States). The normal distribution of continuous data was checked using the Kolmogorov-Smirnov test. Those continuous data conforming to the normal distribution were presented as means ± SD and analyzed using Student's *t*-test (two groups) or ANOVA (more than two groups). Otherwise, they were presented as medians (ranges) and analyzed using the Wilcoxon-Mann-Whitney *U*-test (two groups) or the Kruskal-Wallis analysis of variance (more than two groups). Categorical data were displayed as numbers (percent). Pearson's correlation analysis was used to determine the pairwise correlations among KAP scores. A multivariate logistic regression analysis was performed to determine the independent factors relevant to the KAP score. Variables with *P*-values less than 0.20 in the univariate analysis were included in the multivariate logistic analysis. Two-sided *P*-values below 0.05 were regarded as statistically significant.

RESULTS

The present study included a total of 353 valid questionnaires. Most of the participants were male (63.5%), aged 20-30 (32.9%) years. The other social-demographic data are presented in Table 1. The mean knowledge, attitude, and practice scores were 10.05 ± 3.46 (possible range: 0-14), 41.58 ± 5.23(possible range: 0-56), and 44.20 ± 7.39 (possible range: 0-56), respectively, indicating good knowledge, positive attitude, and proactive practice toward IBD (Table 1).

The knowledge items with the lowest score were K2 (21.0%, "At present, and many factors such as heredity, immunity, environment, and microorganisms are involved in the pathogenesis of the disease"), K11 (42.2%, "There are no side effects under the therapy of glucocorticoids, *etc.*"), K4 (60.1%, "Extraintestinal manifestations of IBD include oral ulcers, joint injury, skin injury, eye lesions, hepatobiliary diseases, *etc.*"), and K13 (65.7%, "All patients with IBD cannot normally absorb the nutrients they intake") (Table 2). The attitude item with the lowest score was A8 ("I think that treatment can be stopped when the colonoscopy shows mucosal healing *i.e.*, complete healing of colonic erosions and ulcers") (Table 3). The practice item with the lowest score was P11 ("I will use a diet diary to identify foods that may cause discomforts such as abdominal pain or diarrhea and try to avoid them in my future diet"). In addition, 98.0% of the participants were willing to stop smoking and drinking (Table 4).

The knowledge score was found to be related to the attitude score (r = 0.371, P < 0.001) and practice (r = 0.100, P < 0.001) score, respectively. The attitude score was related to the practice score (r = 0.452, P < 0.001) (Table 5). Moreover, multivariate logistic regression analysis suggested that aged 30-40 years [odds ratio (OR) = 4.06, 95% confidence interval (CI): 1.04-15.82, P = 0.043], middle school education (OR = 3.98, 95%CI: 1.29-12.33, P = 0.017), high school/technical secondary school education (OR = 14.06, 95%CI: 3.92-50.38, P < 0.001), and junior college/bachelor's degree and above education (OR = 15.20, 95%CI: 4.15-55.650, P < 0.001) were independently linked with the knowledge score (Table 6). The knowledge score (OR = 1.23, 95%CI: 1.11-1.36, P < 0.001) was independently associated with the attitude score (Table 7). In addition, the attitude score (OR = 1.20, 95%CI: 1.11-1.30, P < 0.001) had an independent effect on the practice score (Table 8).

Table 1 Characteristics of the participants

Variables	*n* (%)	Knowledge		Attitude		Practice	
		mean ± SD	*P* value	mean ± SD	*P* value	mean ± SD	*P* value
Total	353	10.05 ± 3.46		41.58 ± 5.23		44.20 ± 7.39	
Gender			0.468		0.830		0.077
Male	224 (63.5)	9.95 ± 3.57		41.62 ± 5.02		44.72 ± 7.35	
Female	129 (36.5)	10.2 ± 3.28		41.50 ± 5.59		43.28 ± 7.40	
Age, yr (10 cases missing)			< 0.001		0.142		0.886
≤ 20	41 (11.6)	9.46 ± 3.87		40.95 ± 5.13		44.00 ± 8.15	
20-30	116 (32.9)	10.87 ± 2.94		42.41 ± 5.25		44.37 ± 7.40	
30-40	85 (24.1)	10.56 ± 3.03		41.81 ± 5.24		43.89 ± 6.92	
> 40	101 (28.6)	9.02 ± 3.91		40.88 ± 5.21		44.70 ± 7.27	
Ethnicity (1 case missing)			0.011		0.028		0.609
Han	341 (96.6)	10.13 ± 3.39		41.69 ± 5.16		44.25 ± 7.41	
Minorities	11 (3.1)	7.45 ± 4.89		38.18 ± 6.51		43.09 ± 6.85	
Residence			0.006		0.059		0.002
Rural	149 (42.2)	9.38 ± 3.72		44.18 ± 5.19		43.76 ± 7.76	
City	122 (34.6)	10.66 ± 3.05		42.48 ± 5.05		45.97 ± 6.73	
Suburb/urban-rural combination	82 (23.2)	10.35 ± 3.85		40.95 ± 5.45		42.35 ± 7.13	
Education			< 0.001		0.003		0.089
Primary school and below	25 (7.1)	6.92 ± 3.53		40.00 ± 4.97		42.88 ± 7.21	
Middle school	67 (19.0)	7.99 ± 4.17		39.99 ± 5.82		42.40 ± 8.01	
High school/technical secondary school	84 (23.8)	10.25 ± 2.88		41.42 ± 4.83		44.55 ± 6.98	
Junior college/bachelor's degree and above	177 (50.1)	11.18 ± 2.75		42.47 ± 5.05		44.89 ± 7.29	
Work status			< 0.001		0.002		0.012
Employed	185 (52.4)	10.79 ± 2.96		42.39 ± 4.90		45.13 ± 6.83	
Other	168 (47.6)	9.23 ± 3.78		40.68 ± 5.44		43.17 ± 7.85	
Monthly per capita income			< 0.001		0.003		0.074
< 5000	173 (49.0)	9.32 ± 3.87		40.61 ± 5.20		43.29 ± 7.95	
5000-10000	104 (29.5)	10.88 ± 2.52		42.37 ± 4.96		45.21 ± 6.59	
> 10000	76 (21.5)	10.55 ± 3.27		42.68 ± 5.32		44.87 ± 6.93	
Marital status			0.029		0.939		0.201
Unmarried or other	157 (44.5)	10.50 ± 3.21		41.60 ± 5.25		44.76 ± 7.40	
Married	196 (55.5)	9.69 ± 3.62		41.56 ± 5.23		43.74 ± 7.37	
Smoking habit			0.163		0.386		0.202

No (no smoking)	282 (79.9)	10.18 ± 3.33		41.45 ± 5.13		43.94 ± 7.18	
Yes (smoking or used to smoke)	71 (20.1)	9.54 ± 3.92		42.06 ± 5.63		45.20 ± 8.14	
Drinking habit			0.461		0.744		0.372
No (no drinking)	240 (68.0)	10.14 ± 3.40		41.51 ± 5.27		43.95 ± 7.36	
Yes (drinking or used to drink)	113 (32.0)	9.85 ± 3.59		41.71 ± 5.16		44.71 ± 7.47	
Medical insurance type (multiple choices)							
Basic medical insurance for urban employees	187 (53.0)	10.68 ± 3.06	< 0.001	43.62 ± 4.98	< 0.001	44.79 ± 7.14	0.108
New cooperative medical insurance	112 (31.7)	9.16 ± 4.02	0.001	54.18 ± 5.45	0.001	43.34 ± 7.88	0.138
Basic medical insurance for urban residents	62 (17.6)	9.18 ± 3.77	0.029	55.13 ± 4.87	0.460	44.56 ± 6.71	0.666
Commercial insurance	23 (6.5)	10.74 ± 3.37	0.323	56.30 ± 4.76	0.490	45.61 ± 7.24	0.343
No insurance	3 (0.8)	12.33 ± 1.15	0.252	52.00 ± 4.58	0.235	34.67 ± 9.29	0.025
Which IBD			< 0.001		0.005		0.553
Ulcerative colitis	133 (37.7)	9.16 ± 3.69		54.57 ± 5.06		43.89 ± 7.55	
Crohn's disease	220 (62.3)	10.59 ± 3.21		56.18 ± 5.25		44.38 ± 7.30	
Duration of IBD			0.995		0.948		0.248
< 1 yr	239 (67.7)	10.05 ± 3.46		55.58 ± 5.17		44.65 ± 7.36	
1-2 yr	59 (16.7)	10.08 ± 3.43		55.41 ± 5.30		43.17 ± 7.72	
> 2 yr	55 (15.6)	10.02 ± 3.56		55.73 ± 5.59		43.33 ± 7.09	
Ostomy			0.014		0.088		0.621
Yes	27 (7.6)	8.48 ± 4.37		53.93 ± 5.95		43.52 ± 10.05	
No	326 (92.4)	10.18 ± 3.53		55.71 ± 5.15		44.25 ± 7.14	
Comorbidities			0.463		0.064		0.004
Yes	59 (16.7)	9.75 ± 3.72		54.42 ± 5.11		41.71 ± 7.32	
No	294 (83.3)	10.11 ± 3.41		55.81 ± 5.23		44.64 ± 7.28	
Family history of IBD			0.588		0.991		0.392
Yes	9 (2.5)	10.67 ± 4.21		55.56 ± 4.98		42.11 ± 6.43	
No	344 (97.5)	10.03 ± 3.45		55.58 ± 5.24		44.25 ± 7.41	
Surgical history			0.340		0.487		0.894
Yes	165 (46.7)	10.24 ± 3.39		55.78 ± 5.10		44.14 ± 7.47	
No	188 (53.3)	9.88 ± 3.53		55.39 ± 5.35		44.24 ± 7.34	
History of drug allergy			0.110		0.120		0.890
Yes	48 (13.6)	10.79 ± 2.73		56.67 ± 5.15		44.33 ± 7.36	
No	305 (86.4)	9.93 ± 3.55		55.40 ± 5.23		44.17 ± 7.41	
What kind of treatment is being received?			0.040		0.004		0.276
5-aminosalicylic acid drugs (*e.g.*, mesalazine)	19 (5.4)	8.63 ± 3.44		55.00 ± 4.99		45.32 ± 8.87	

Glucocorticoids	1 (0.3)	12.00	57.00	50.00
Immunosuppressants (*e.g.*, azathioprine, tacrolimus, cyclosporine, *etc.*)	6 (1.7)	6.50 ± 3.27	51.33 ± 6.38	37.83 ± 9.99
Biological agents (*e.g.*, infliximab, vedolizumab, ustekinumab)	301 (85.3)	10.16 ± 3.49	55.97 ± 5.13	44.33 ± 7.26
Biological agents + immunosuppressants	14 (4.0)	9.93 ± 3.34	51.36 ± 5.58	43.57 ± 6.73
Biological agents + 5-aminosalicylic acid drugs	12 (3.4)	11.25 ± 1.48	53.42 ± 4.36	42.50 ± 6.99

IBD: Inflammatory bowel disease.

Table 2 Knowledge dimension, *n* (%)

Knowledge	Correct	Wrong	Unclear
IBD is a group of chronic, non-specific recurrent intestinal inflammatory diseases, including UC and CD	316 (89.5)	2 (0.6)	35 (9.9)
At present, many factors, such as heredity, immunity, environment, and microorganisms, are involved in the pathogenesis of the disease	74 (21.0)	185 (52.4)	94 (26.6)
Symptoms of IBD can include abdominal pain, diarrhea, bloody stool, anemia, fever, joint swelling, pain, *etc.*	293 (83.0)	13 (3.7)	47 (13.3)
Extraintestinal manifestations of IBD include oral ulcers, joint injury, skin injury, eye lesions, hepatobiliary diseases, *etc.*	212 (60.1)	32 (9.1)	109 (30.9)
IBD often occurs in young adults and is more common between the ages of 20-50 yr	267 (75.6)	16 (4.5)	70 (19.8)
IBD is a lifelong disease, and the patient's condition is prolonged and repeated. At present, there is no specific and effective medicine or method to cure the disease	293 (83.0)	11 (3.1)	49 (13.9)
Colonoscopy and mucosal biopsy are the best methods to establish the diagnosis and assess the disease's severity in patients with IBD	285 (80.7)	6 (1.7)	62 (17.6)
Generally, medical treatment is the main treatment for IBD, but surgical treatment is needed when intestinal obstruction, intestinal perforation, and canceration occur	275 (77.9)	5 (1.4)	73 (20.7)
The treatment of patients with IBD varies widely among individuals, with different classifications and severity of the disease leading to different treatment outcomes and efficacy	299 (84.7)	1 (0.3)	53 (15.0)
Drugs for treating IBD include hormones, aminosalicylic acid drugs, immunosuppressants (azathioprine, methotrexate, *etc.*), and biological agents	274 (77.6)	5 (1.4)	74 (21.0)
There are no side effects after treatment with glucocorticoids, *etc.*	149 (42.2)	39 (11.0)	165 (46.7)
Currently, the biological agents approved for treating IBD in China include infliximab, vidrizumab, and ustekinumab	292 (82.7)	6 (1.7)	55 (15.6)
All patients with IBD can't normally absorb the nutrients they intake	232 (65.7)	60 (17.0)	61 (17.3)
Emotion, smoking, drinking, and other behaviors will not affect IBD	286 (81.0)	36 (10.2)	31 (8.8)

IBD: Inflammatory bowel disease; UC: Ulcerative colitis; CD: Crohn's disease.

DISCUSSION

The findings of our study suggested that Chinese patients with IBD had good knowledge, positive attitudes, and proactive practice toward their disease. Nevertheless, some specific items warranting more education were identified. These outcomes may be useful for the management and self-management of IBD patients in clinical practice.

Several studies revealed misconceptions and relatively poor knowledge in patients with IBD about their disease[16-21]. A study from England published 30 years ago already acknowledged that patients with IBD had poor knowledge regarding their disease but were willing to acquire information[16]. More contemporary data indicated little progress since then, *i.e.*, that the knowledge of patients with IBD toward their disease was poor[17-21], including in New Zealand [17], Canada[18], Israel[19], Poland[20], and South Korea[21]. Surprisingly, in the present study, the patients with IBD showed good KAP toward IBD, but it could be noted that most participants had a junior college/bachelor's degree and above education and were receiving expensive biological agents, thereby suggesting a higher socioeconomic status that could influence the results.

Table 3 Attitude dimension, *n* (%)

Attitude	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
I am confident in the treatment of IBD	158 (44.8)	134 (38.0)	58 (16.4)	3 (0.8)	0
I think patients with IBD need to avoid certain foods	186 (52.7)	144 (40.8)	22 (6.2)	1 (0.3)	0
I think that patients with IBD combined with malnutrition need to use a combination of intestinal and extra-intestinal nutrition support according to the disease situation if necessary	171 (48.4)	151 (42.8)	29 (8.2)	1 (0.3)	1 (0.3)
I think scientific dietary guidance and management are key to managing IBD	193 (54.7)	141 (39.9)	18 (5.1)	1 (0.3)	0
I think developing a specific treatment plan for IBD needs to be tailored to the individual's situation and developed jointly by the IBD medical specialist and the patient	208 (58.9)	129 (36.5)	15 (4.2)	1 (0.3)	0
I think the adjustment of IBD medication needs to be carried out under the guidance of specialists, and patients should not adjust their own medication	228 (64.6)	112 (31.7)	13 (3.7)	0	0
I believe that during IBD medication, patients need to monitor the side effects of their medication and provide timely feedback to their specialists	216 (61.2)	128 (36.3)	9 (2.5)	0	0
I think treatment can be stopped when the colonoscopy shows mucosal healing (*i.e.*, complete healing of colonic erosions and ulcers)	37 (10.5)	47 (13.3)	92 (26.1)	134 (38.0)	43 (12.2)
I think the early application of biologics, in conjunction with specialist advice, will allow early control of disease activity to change the course of the disease and minimize complications and disability in the bowel	171 (48.4)	147 (41.6)	30 (8.5)	3 (0.8)	2 (0.6)
I think patients with IBD should reduce their intake of saturated fatty acids (animal oil, cream, fatty meats, meat soups, *etc.*)	137 (38.8)	156 (44.2)	52 (14.7)	7 (2.0)	1 (0.3)
I think that IBD patients should try to drink plain hot water and freshly squeezed juices and need to avoid strong tea, sugary drinks, coffee, alcohol, *etc.*	177 (50.1)	148 (41.9)	24 (6.8)	2 (0.6)	2 (0.6)
I think the IBD disease has obviously increased the family's financial burden	172 (48.7)	133 (37.7)	42 (11.9)	5 (1.4)	1 (0.3)
I think I can get married, get pregnant, and give birth normally if my IBD disease is controlled	125 (35.4)	166 (47.0)	52 (14.7)	8 (2.3)	2 (0.6)
I think IBD has affected my normal work, study, and social interaction	98 (27.8)	134 (38.0)	86 (24.4)	30 (8.5)	5 (1.4)

IBD: Inflammatory bowel disease.

The present investigation also demonstrated that age and educational attainment were independently associated with knowledge scores. Specific knowledge items that need improvement include the etiology of IBDs, the possible extra-intestinal manifestations of IBDs, the side effects of glucocorticoids, and nutrient absorption. Even though the other knowledge items had high scores, none scored above 90%, indicating they would benefit from additional instruction. Furthermore, knowledge was the only factor independently associated with the attitude score, and attitude was the only factor independently connected with the practice score. Hence, improving the knowledge of patient about IBDs should enhance their KAP. Since a proper KAP of IBDs has been associated with better IBD outcomes[15], improving the KAP can improve patient outcomes, given that self-management is at the core of IBD management[12,13].

Still, patients obtain knowledge primarily from available resources (books, the internet, newspapers, *etc.*), their social network, and healthcare professionals. A study highlighted variable access to high-quality information on IBD-related nutrition[26], and nutrition is a major factor influencing the intestinal microflora and the outcomes of IBDs[27-29]. Furthermore, a study in New Zealand showed that the KAP of IBD in the general population was also low[30,31], suggesting that patient education is deficient or ineffective since the patients with IBD have poor KAP. Healthcare providers are the primary source of reliable patient information, but studies unveiled that the KAP of IBD among healthcare providers was also low[32,33]. Having limited knowledge about a disease can impede the spread of accurate information. Therefore, previous studies suggest that priority should be placed on educating patients, healthcare professionals, and the general public.

There are some limitations in this study. It was conducted at a single institution, limiting its applicability to other hospitals in China. The questionnaire was designed by local investigators and was probably influenced by local policies and guidelines, further restricting the exportability of the questionnaire. The study has local scope, and the results cannot be extrapolated to other populations, which makes similar studies necessary in other locations. The study population shows high education and use of biological products, which suggests a selection bias. KAP surveys represent the situation of a specific population at a precise time point. Therefore, studies in other populations and time might be necessary to examine the actual KAP situation in China and the effect of education. Finally, all KAP surveys were susceptible to

Table 4 Practice dimension, *n* (%)

Practice	Always	Often	Sometimes	Seldom	Never
I will actively cooperate with the medical staff for my treatment and nursing	244 (69.1)	93 (26.3)	15 (4.2)	1 (0.3)	0
I will communicate with specialists regularly and follow up regularly	166 (47.0)	116 (32.9)	62 (17.6)	9 (2.5)	0
I will vent my bad emotions correctly, such as through exercise relaxation, music relaxation, and implied adjustment, to relieve mental stress	106 (30.0)	125 (35.4)	92 (26.1)	25 (7.1)	5 (1.4)
I will communicate with family members, close friends, and patients and gain encouragement and emotional support	94 (26.6)	117 (33.1)	92 (26.1)	41 (11.6)	9 (2.5)
	Yes, *n* (%)		No, *n* (%)		
I will take care to quit smoking and drinking	346 (98.0)		7 (2.0)		
I will take care to avoid staying up late and overworking	120 (34.0)	119 (33.7)	87 (24.6)	23 (6.5)	4 (1.1)
I will take care to choose appropriate physical exercise according to my physical condition	96 (27.2)	95 (26.9)	105 (29.7)	50 (14.2)	7 (2.0)
If there is an ostomy, I will go to an IBD specialist for standard treatment	336 (95.2)	17 (4.8)	0	0	0
If I am treated with biological agents, I will pay attention to monitoring the related side effects	179 (50.7)	107 (30.3)	52 (14.7)	11 (3.1)	4 (1.1)
If a food allergy is identified, I will take care to avoid it in my daily diet	210 (59.5)	105 (29.7)	30 (8.5)	5 (1.4)	3 (0.8)
I will use a "diet diary" to identify foods that may cause discomfort, such as abdominal pain or diarrhea, and try to avoid them in my future diet	106 (30.0)	94 (26.6)	67 (19.0)	42 (11.9)	44 (12.5)
I will improve my understanding of diseases and treatment through WeChat groups, networks, and popular science lectures	100 (28.3)	85 (24.1)	104 (29.5)	52 (14.7)	12 (3.4)
I will insist on taking medicine or receiving infusion treatment of biological agents as prescribed by my physician	247 (70.0)	86 (24.4)	16 (4.5)	3 (0.8)	1 (0.3)
I will encourage and help other people with IBD as much as I can	135 (38.2)	78 (22.1)	93 (26.3)	37 (10.5)	10 (2.8)

IBD: Inflammatory bowel disease.

Table 5 Correlation analysis

	Knowledge dimension	Attitude	Practice
Knowledge dimension	1		
Attitude	0.371 ($P < 0.001$)	1	
Practice	0.100 ($P < 0.001$)	0.452 ($P < 0.001$)	1

Table 6 Univariate and multivariate analysis of knowledge

Variables	Univariate analysis		Multivariate analysis	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Gender				
Male	1.09 (0.62-1.92)	0.751		
Female	Ref			
Age				
≤ 20	Ref		Ref	
20-30	2.80 (1.10-7.09)	0.030	3.01 (0.97-9.38)	0.057
30-40	2.17 (0.84-5.63)	0.111	4.06 (1.04-15.82)	0.043
> 40	0.76 (0.33-1.75)			

Ethnicity				
Han	3.83 (1.13-12.94)	0.031	3.70 (0.80-16.97)	0.093
Minorities	Ref		Ref	
Residence				
Rural	Ref		Ref	
City	3.08 (1.56-6.07)	0.001	1.69 (0.74-3.84)	0.213
Suburb/urban-rural combination	1.95 (0.97-3.90)	0.060	1.33 (0.58-3.03)	0.496
Education				
Primary school and below	Ref			
Middle school	2.99 (1.15-7.76)	0.025	3.98 (1.29-12.33)	0.017
High school/technical secondary school	11.80 (4.20-33.17)	< 0.001	14.06 (3.92-50.38)	< 0.001
Junior college/bachelor's degree and above	20.70 (7.75-55.28)	< 0.001	15.20 (4.15-55.65)	< 0.001
Work status				
Employed	4.07 (2.23-7.41)	< 0.001	1.34 (0.63-2.85)	0.444
Other	Ref		Ref	
Monthly per capita income				
< 5000	Ref		Ref	
5000-10000	3.94 (1.84-8.43)	< 0.001	2.04 (0.81-5.18)	0.133
> 10000	2.46 (1.17-5.18)	0.018	0.90 (0.34-2.36)	0.823
Marital status				
Unmarried or other	Ref		Ref	
Married	0.61 (0.35-1.07)	0.083	0.85 (0.33-2.16)	0.734
Smoking habit				
No (no smoking)	Ref			
Yes (smoking or used to smoke)	0.60 (0.33-1.12)	0.10		
Drinking habit				
No (no drinking)	Ref			
Yes (drinking or used to drink)	0.85 (0.49-1.50)	0.584		
What kind of IBD is being diagnosed				
Ulcerative colitis	0.50 (0.29-0.85)	0.011	0.57 (0.28-1.18)	0.132
Crohn's disease	Ref		Ref	
Duration of IBD				
< 1 yr	1.14 (0.54-2.39)	0.729		
1-2 yr	0.98 (0.39-2.45)	0.964		
> 2 yr	Ref			
Ostomy?				
Yes	0.51 (0.21-1.23)	0.135		
No	Ref			
Comorbidities				
Yes	0.62 (0.32-1.19)	0.149		
None	Ref			
Family history of IBD				
Yes	1.86 (0.23-15.16)	0.560		

No	Ref	
Surgical history		
Yes	1.15 (0.67-1.97)	0.613
No	Ref	
History of drug allergy		
Yes	1.40 (0.60-3.29)	0.433
No	Ref	
What kind of treatment is being received?		
5-aminosalicylic acid drugs (*e.g.*, mesalazine)	Ref	
Glucocorticoids	-	-
Immunosuppressants (*e.g.*, azathioprine, tacrolimus, cyclosporine, *etc.*)	0.46 (0.07-2.99)	0.418
Biological agents (*e.g.*, infliximab, vedolizumab, ustekinumab)	2.11 (0.77-5.80)	0.148
Biological agents + immunosuppressants	1.69 (0.34-8.40)	0.520
Biological agents + 5-aminosalicylic acid drugs	-	-

IBD: Inflammatory bowel disease.

Table 7 Univariate and multivariate analysis of attitude

Variables	Univariate analysis		Multivariate analysis	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Knowledge score (as continuous variables)	1.24 (1.14-1.34)	< 0.001	1.23 (1.11-1.36)	< 0.001
Gender				
Male	1.42 (0.73-2.74)	0.300		
Female	Ref			
Age				
≤ 20	Ref			
20-30	1.49 (0.52-4.25)	0.461		
30-40	1.29 (0.43-3.82)	0.651		
> 40	1.27 (0.44-3.65)	0.656		
Ethnicity				
Han	4.69 (1.31-16.80)	0.017	3.21 (0.66-15.59)	0.149
Minorities	Ref		Ref	
Residence				
Rural	Ref		Ref	
City	2.47 (1.06-5.76)	0.037	1.63 (0.61 4.32)	0.329
Suburb/urban-rural combination	1.12 (0.51-2.44)	0.779	0.98 (0.39-2.47)	0.968
Education				
Primary school and below	Ref		Ref	
Middle school	0.95 (0.30-2.97)	0.925	0.85 (0.23-3.08)	0.803
High school/technical secondary school	2.37 (0.70-8.05)	0.165	0.87 (0.22-3.49)	0.841
Junior college/bachelor's degree and above	2.91 (0.95-8.94)	0.062	0.82 (0.20-3.26)	0.774
Work status				
Employed	2.65 (1.32-5.30)	0.006	1.54 (0.64-3.70)	0.338

Other	Ref		Ref	
Monthly per capita income				
< 5000	Ref		Ref	
5000-10000	2.22 (0.97-5.09)	0.060	1.10 (0.42-2.89)	0.850
> 10000	2.16 (0.85-5.46)	0.105	1.06 (0.35-3.17)	0.924
Marital status				
Unmarried or other	Ref			
Married	0.97 (0.51-1.88)	0.937		
Smoking habit				
No (no smoking)	Ref			
Yes (smoking or used to smoke)	0.88 (0.40-1.94)	0.755		
Drinking habit				
No (no drinking)	Ref			
Yes (drinking or used to drink)	1.16 (0.57-2.36)	0.689		
What kind of IBD is being diagnosed				
Ulcerative colitis	0.75 (0.39-1.44)	0.383		
Crohn's disease	Ref			
Duration of IBD				
< 1 yr	0.92 (0.36-2.35)	0.866		
1-2 yr	0.91 (0.29-2.90)	0.873		
> 2 yr	Ref			
Ostomy?				
Yes	0.55 (0.19-1.53)	0.250		
No	Ref			
Comorbidities				
Yes	0.68 (0.31-1.51)	0.341		
None	Ref			
Family history of IBD				
Yes	1.05 (0.13-8.64)	0.962		
No	Ref			
Surgical history				
Yes	1.14 (0.59-2.19)	0.698		
No	Ref			
History of drug allergy				
Yes	1.52 (0.52-4.47)	0.448		
No	Ref			
What kind of treatment is being received?				
5-aminosalicylic acid drugs (*e.g.*, mesalazine)	Ref		Ref	
Glucocorticoids	-	-	-	-
Immunosuppressants (*e.g.*, azathioprine, tacrolimus, cyclosporine, *etc.*)	0.24 (0.02-2.22)	0.206	0.38 (0.03-4.43)	0.438
Biological agents (*e.g.*, infliximab, vedolizumab, ustekinumab)	1.10 (0.24-5.02)	0.899	0.88 (0.17-4.53)	0.878
Biological agents + immunosuppressants	0.21 (0.03-1.32)	0.096	0.16 (0.02-1.17)	0.070
Biological agents + 5-aminosalicylic acid drugs	0.35 (0.05-2.51)	0.298	0.17 (0.02-1.51)	0.113

IBD: Inflammatory bowel disease.

Table 8 Univariate and multivariate analysis of practice

Variables	Univariate analysis		Multivariate analysis	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Knowledge score (as continuous variables)	1.10 (1.02-1.19)	0.020	0.96 (0.87-1.06)	0.412
Attitude score (as continuous variables)	1.21 (1.13-1.30)	< 0.001	1.20 (1.11-1.30)	< 0.001
Gender				
Male	1.62 (0.86-3.04)	0.134		
Female	Ref			
Age				
≤ 20	Ref			
20-30	1.25 (0.45-3.50)	0.672		
30-40	1.65 (0.53-5.11)	0.386		
> 40	1.16 (0.41-3.30)	0.780		
Ethnicity				
Han	0.65 (0.14-3.11)	0.589		
Minorities	Ref			
Residence				
Rural	Ref		Ref	
City	2.41 (1.08-5.40)	0.033	2.01 (0.80-5.04)	0.139
Suburb/urban-rural combination	1.12 (0.53-2.38)	0.768	1.12 (0.49-2.58)	0.788
Education				
Primary school and below	Ref			
Middle school	0.66 (0.20-2.22)	0.503		
High school/technical secondary school	2.10 (0.56-7.84)	0.272		
Junior college/bachelor's degree and above	1.58 (0.49-5.11)	0.441		
Work status				
Employed	2.76 (1.41-5.40)	0.003	1.93 (0.88-4.21)	0.099
Other	Ref		Ref	
Monthly per capita income				
< 5000	Ref		Ref	
5000-10000	2.42 (1.06-5.51)	0.036	1.31 (0.51-3.33)	0.578
> 10000	1.71 (0.74-3.94)	0.207	0.86 (0.32-2.28)	0.755
Marital status				
Unmarried or other	Ref			
Married	0.65 (0.34-1.25)	0.200		
Smoking habit				
No (no smoking)	Ref			
Yes (smoking or used to smoke)	0.86 (0.41-1.84)	0.706		
Drinking habit				
No (no drinking)	Ref			

Yes (drinking or used to drink)	1.18 (0.60-2.35)	0.631		
What kind of IBD is being diagnosed				
Ulcerative colitis	0.80 (0.43-1.52)	0.501		
Crohn's disease	Ref			
Duration of IBD				
< 1 yr	1.39 (0.59-3.27)	0.445		
1-2 yr	0.74 (0.27-2.01)	0.558		
> 2 yr	Ref			
Ostomy				
Yes	0.62 (0.22-1.72)	0.354		
No	Ref			
Comorbidities				
Yes	0.43 (0.21-0.88)	0.022	0.50 (0.23-1.09)	0.082
None	Ref		Ref	
Family history of IBD				
Yes	-	-		
No	Ref			
Surgical history				
Yes	1.11 (0.59-2.09)	0.741		
No	Ref			
History of drug allergy				
Yes	0.83 (0.35-1.99)	0.682		
No	Ref			
What kind of treatment is being received?				
5-aminosalicylic acid drugs (*e.g.*, mesalazine)	Ref			
Glucocorticoids	-	-		
Immunosuppressants (*e.g.*, azathioprine, tacrolimus, cyclosporine, *etc.*)	0.19 (0.02-1.41)	0.104		
Biological agents (*e.g.*, infliximab, vedolizumab, ustekinumab)	1.38 (0.38-4.97)	0.622		
Biological agents + immunosuppressants	2.44 (0.23-26.30)	0.463		
Biological agents + 5-aminosalicylic acid drugs	0.94 (0.13-6.63)	0.948		

IBD: Inflammatory bowel disease.

social desirability bias, in which participants may have been more likely to provide the expected response than the actual answer[34].

CONCLUSION

In conclusion, this study suggests that Chinese patients with IBD have good knowledge, positive attitudes, and active practice toward their disease. Nevertheless, some specific items warranting more education were identified, especially regarding the etiology and contributing factors to the disease, extraintestinal manifestations, glucocorticoid side effects, and nutrient absorption.

ARTICLE HIGHLIGHTS

Research background

The management of inflammatory bowel disease (IBD) necessitates the adoption of healthy lifestyle habits, which requires proper knowledge, attitudes, and practice of the specific lifestyle routines to implement. However, patients with IBD generally have poor knowledge, attitude, and practice (KAP) of their disease, while the data from China are lacking.

Research motivation

The motivation of this study is to help healthcare providers to improve the patient's self-management of IBD.

Research objectives

The object of this study is to investigate the KAP of patients with IBD toward their disease in Zhejiang Province, China.

Research methods

Self-designed questionnaires were administered to the participants through WeChat on the SoJump platform (https://www.wjx.cn/app/survey.aspx). Pearson's correlation analysis was used to determine the pairwise correlations among KAP scores. A multivariate logistic regression analysis was further performed to determine the independent factors associated with their KAP scores.

Research results

A total of 353 patients (224 males) with IBD completed the questionnaires. Their mean KAP scores were 10.05 ± 3.46 (possible range: 0-14), 41.58 ± 5.23 (possible range: 0-56), 44.20 ± 7.39 (possible range: 0-56), respectively, indicating good knowledge, positive attitude, and proactive practice toward IBD. Age and education were independently associated with their KAP.

Research conclusions

Chinese patients with IBD might have good knowledge, a positive attitude, and proactive practice toward their disease. Nevertheless, some specific items warranting more education were identified, especially regarding the etiology and contributing factors to the disease, extraintestinal manifestations, glucocorticoid side effects, and nutrient absorption.

Research perspectives

The findings of this study may be useful for the management and self-management of IBD patients in clinical practice.

FOOTNOTES

Author contributions: Fang LY and Guo XR carried out the study and participated in collecting data; Shao XX drafted the manuscript; Wang WZ and Shi RX performed the statistical analysis and participated in its design; Lin DP participated in the acquisition, analysis, and interpretation of data; and all authors read and approved the final manuscript.

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ORCID number: Dao-Po Lin 0000-0003-0785-7675.

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ORIGINAL ARTICLE

Observational Study

Helicobacter pylori infection in Xinjiang Uyghur Autonomous Region: Prevalence and analysis of related factors

Yu-Hua Peng, Xue Feng, Zhong Zhou, Lei Yang, Yun-Fei Shi

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Yu-Hua Peng, Zhong Zhou, Department of Pathology, Traditional Chinese Medical Hospital of Xinjiang Uygur Autonomous Region, The Affiliated Hospital of Traditional Chinese Medicine of Xinjiang Medical University, Urumqi 830000, Xinjiang Uygur Autonomous Region, China

Xue Feng, Department of Laboratory Medicine, Traditional Chinese Medical Hospital of Xinjiang Uygur Autonomous Region, The Affiliated Hospital of Traditional Chinese Medicine of Xinjiang Medical University, Urumqi 830000, Xinjiang Uygur Autonomous Region, China

Lei Yang, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Beijing Office for Cancer Prevention and Control, Peking University Cancer Hospital & Institute, Beijing 100142, China

Yun-Fei Shi, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Pathology, Peking University Cancer Hospital & Institute, Beijing 100142, China

Corresponding author: Yun-Fei Shi, MD, PhD, Doctor, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Pathology, Peking University Cancer Hospital & Institute, No. 52 Fucheng Road, Haidian District, Beijing 100142, China. shiyunfei@bjmu.edu.cn

Abstract

BACKGROUND

^{14}C urea breath test (^{14}C UBT) and immunohistochemical staining (IHC) are widely used for detection *Helicobacter pylori* (*H. pylori*) infection with different sensitivity, and there is a difference in *H. pylori* infection rate in Uyghur and Han ethnic groups. Both need large cohort studies to evaluate the differences more accurately.

AIM

To analyze the difference between ^{14}C UBT and IHC for *H. pylori* detection in Xinjiang Uyghur Autonomous Region and the difference between Uyghur and Han populations.

METHODS

There were 3944 cases of *H. pylori* infection detected by both IHC and ^{14}C UBT at the same time (interval < 1 wk, with sampling site including gastric antrum, selected from 5747 patients). We compared the sensitivity of ^{14}C UBT and IHC. We

also compared 555 pairs of Han/Uyghur cases (completely matched for gender and age) for their *H. pylori* infection rates. The overall *H. pylori* infection rate of all 5747 cases and the correlation with other clinicopathological data were also further analyzed. SPSS V23.0 software was used for statistical analysis.

RESULTS

The sensitivity was 94.9% for ^{14}C UBT and 65.1% for IHC, which was a significant difference (n = 3944, P < 0.001). However, among those cases negative for *H. pylori* by ^{14}C UBT (detection value ≤ 100), 4.8% were positive by IHC. Combining both methods, the overall *H. pylori* infection rate was 48.6% (n = 5747), and differences in gender, age group, ethnicity and region of residence significantly affected the *H. pylori* positive rates. According to age group (Han/Uyghur), the positive rates were ≤ 30 years (62.2%/100.0%), 31-40 years (45.2%/85.7%), 41-50 years (47.2%/79.2%), 51-60 years (44.6%/76.1%), 61-70 years (40.9%/68.2%), 71-80 years (41.7%/54.1%) and ≥ 81 years (42.9%/NA). The *H. pylori* infection rates of Han/Uyghur paired cases were 41.4% and 73.3%, which was a significant difference (P < 0.001) (555 pairs). *H. pylori* positivity was significantly related to moderate-severe grade 2-3 chronic/active gastritis and intestinal metaplasia (all P < 0.05).

CONCLUSION

The sensitivity of ^{14}C UBT was significantly higher, but combined application can still increase the accuracy. The prevention *H. pylori* should be emphasized for Uygur and young people.

Key Words: *Helicobacter pylori*; Immunohistochemistry; ^{14}C urea breath test; Han; Uyghur; Xinjiang Uyghur Autonomous Region

Core Tip: The sensitivity of ^{14}C urea breath test (^{14}C UBT) for detecting *Helicobacter pylori* (*H. pylori*) is significantly higher than that of immunohistochemistry (IHC) with endoscopy specimens. Combination of ^{14}C UBT and IHC is necessary to improve detection accuracy, and increasing the number of biopsy specimens (≥ 2) can improve the positive rate significantly. The overall rate of *H. pylori* infection in Xinjiang Uyghur Autonomous Region was higher than in previous studies. *H. pylori* infection was more prevalent in the Uyghur population and the infection rate decreased as age increased. Therefore, the prevention and intervention of *H. pylori* infection in Xinjiang Uyghur Autonomous Region should emphasize Uyghur and young people.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a Gram-negative bacterium that mainly parasitizes the oral cavity, stomach, and duodenum after infection[1]. It was first isolated and cultured from patients with gastritis by Warren and Marshall[2] in Australia. It was extensively studied and reported that the vast majority of gastric and duodenal ulcers are associated with *H. pylori* infection, which is also associated with gastric cancer. *H. pylori* has been classified by World Health Organization as a class I carcinogen[3]. Diseases related to *H. pylori* are infectious as emphasized by the Kyoto Consensus [4]. It is known that transmission pathway of *H. pylori* includes fecal-oral and oral-oral transmission[1,5].

The Xinjiang Uyghur Autonomous Region of China has a vast territory and is inhabited by multiple ethnic groups; the main two being Han and Uyghur. Due to the differences in climate, lifestyle, dietary habits, and medical and health conditions, there are differences in *H. pylori* infection status. Although there are some previous reports from Chinese journals, the studies involved small series or their detection methods were not sufficiently sensitive[6-9].

The most important way to more accurately investigate the infection rate of *H. pylori* is to use a more accurate method of detection. Clinical *H. pylori* testing is divided into noninvasive and invasive types[10-12]. The noninvasive tests are mainly carried out in the endoscopy rooms or laboratories of various medical institutions, and the highly recommended method is the ^{14}C urea breath test (^{14}C UBT) test. For the invasive tests, detection of *H. pylori* is through gastroscopic biopsy of pathological tissue. Previous studies have shown that immunohistochemical staining to detect *H. pylori* infection has the highest sensitivity and specificity among all histopathological methods for endoscopic biopsies[13-15]. At present, the consensus is that both immunohistochemistry (IHC) and ^{14}C UBT detection of *H. pylori* infection can be used for diagnosing *H. pylori* infection[16-18]. However, there is controversy in the literature about the sensitivity of IHC and ^{14}C UBT. One study (n = 150) reported that UBT was more sensitive[19] but another study (n = 239) reported that IHC had higher sensitivity[20]. Larger studies are needed to compare the differences between ^{14}C UBT and IHC for detection

of *H. pylori* infection.

Thus, we retrospectively studied a large series of patients who underwent both ^{14}C UBT and IHC and on histopathological diagnosed gastroscopy specimens between January 2019 and February 2021 and through gastroscopy at our hospital in Urumqi. We aimed to investigate the differences between the two detection methods; evaluate *H. pylori* infection rate in Xinjiang Uyghur Autonomous Region; and establish whether there were differences in *H. pylori* infection due to factors such as age, gender, region, and especially, ethnic group (Uyghur and Han). It is hoped that our study might provide more accurate data about the difference in the two widely used *H. pylori* detection methods, and would be useful for the detection, prevention and intervention of *H. pylori* infection in Xinjiang Uyghur Autonomous Region.

MATERIALS AND METHODS

Patients and data collection criteria

A retrospective control study was conducted among patients who went to the Traditional Chinese Medical Hospital of Xinjiang Medical University between March 2019 and February 2021 due to gastrointestinal discomfort for which they underwent pathological examination of gastric biopsy tissue. The screening criteria were as follows: (1) All enrolled patients also underwent ^{14}C UBT; (2) No history of medication (such as antibiotics, proton pump inhibitors, and bismuth-based agents) that affected *H. pylori* activity, or a positive detection rate within 1 mo before the examination; and (3) Complete clinical information. This study met the ethical requirements of the Ethics Committee of Traditional Chinese Medical Hospital of Xinjiang Medical University.

Pathological features

H. pylori infection was detected using hematoxylin and eosin (H&E) staining, IHC staining, and ^{14}C UBT.

H&E staining of paraffin-embedded tissue

All specimens were fixed with 10% neutral formaldehyde solution, routinely dehydrated, embedded, and cut into 4-5 μm sections. We used an automated staining machine (LEICA Company, AUTOSTAINER) for H&E staining.

IHC staining

IHC staining was performed using a BENCHMARK-XT automatic staining machine (Roche) with *H. pylori* antibody working solution (MX104 mouse antihuman monoclonal antibody; Fuzhou Maixin Company), and the positive signal was stained with DAB. Both positive and negative controls were established. The positive control was confirmed to be *H. pylori* positive gastric biopsy tissue with plenty of *H. pylori* (staining > 100 bacterial bodies/*H. pylori*, F). The negative control used a blank antibody solution instead of the first antibody working solution.

Interpretation of pathological results

H&E staining: Pathological diagnosis and gastritis grading (including grading of chronic gastritis, active gastritis, atrophy, and intestinal metaplasia). Classification: Grade 0 (none), grade 1 (mild), grade 2 (moderate), and grade 3 (severe), and statistical analysis was conducted in sequence.

IHC staining sections of H. pylori

Positive/negative results were based on the presence or absence of *H. pylori* bacteria under the microscope. All sections were independently reviewed by two senior pathologists, and for those cases with inconsistency, a third senior pathologist provided the final interpretation.

^{14}C UBT

Patients were tested on an empty stomach or fasting for > 2 h, and the test was positive if ≥ 100 dpm/mmol, and negative if 0-100 dpm/mmol. The ^{14}C urea capsule and related reagents, as well as the *H. pylori* detector, were all provided by Shenzhen Zhonghe Headway Bio-Sci & Tech Co. Ltd.

Criteria for determining positive H. pylori infection

At least one positive result from ^{14}C UBT and IHC is considered as a positive *H. pylori* infection. In the literature, both are the preferred methods for noninvasive and invasive detection; therefore, a positive result with either one can directly diagnose *H. pylori* infection. Both methods have high specificity (default close to 100%). Comparison of the two detection methods in this study focused on the sensitivity aspect. Sensitivity was calculated as: sensitivity = number of true positive cases/(number of true positive cases + number of false negative cases) × 100%.

Grouping and matching and statistical analysis

In order to accurately compare the results of ^{14}C UBT and IHC, it is required that: (1) Gastroscopic pathological examination (H&E and IHC), ^{14}C UBT and the interval between pathological examinations should be < 1 wk; (2) The sampling sites should include the gastric antrum; and (3) The number of samples taken by clinicians should be recorded as it may affect the detection results. A total of 3944 eligible cases were selected from all cases undergoing both ^{14}C UBT and IHC.

We speculated that ethnic differences would be the main factor affecting *H. pylori* infection. We used pathological IHC and ^{14}C UBT, with Uyghur as the case group and Han as the control group, and matched them 1:1. The matching variables included gender and age. A total of 555 fully matched Uyghur and Han cases were obtained, including 249 males and 306 females (male to female ratio of approximately 1:1.23), aged 24-80 years (average 54.1 years, median 48 years), to further investigate the exact impact of ethnic differences on *H. pylori* infection. SPSS version 23.0 was used for statistical analysis and comparison (using χ^2 tests), and $P < 0.05$ represented a statistically significant difference.

RESULTS

Clinical features

A total of 5747 cases were included. Overall, the male to female ratio was 1:1.15 (2672/3075). Age ranged from 19 to 92 years, with a median of 56 years. There were 4925 cases of Han and 822 cases of Uyghur. Divided by the Tianshan Mountains, there were 4919 cases residing in Northern Xinjiang, 508 in Southern Xinjiang, 265 in the Xinjiang Production and Construction Corps (XPCC), and 55 in areas outside Xinjiang Uyghur Autonomous Region. The case information is detailed in Table 1.

Comparative analysis of IHC and ^{14}C UBT for detecting H. pylori infection

For the typical *H. pylori*-positive cases, which were definitively confirmed in the H&E sections (Figure 1A), spiral-shaped or curved bacteria were seen in the superficial mucus secreted from the gastric mucosa, or in the gastric glandular cavity. However, when the number of *H. pylori* was limited or their morphology was atypical (such as coccoid shapes), it was difficult to recognize them (Figures 1B and C). Using *H. pylori* antibodies and IHC can specifically label *H. pylori*. For typical positive cases, *H. pylori* uniformly appeared like small brown rods (Figure 1D). For those cases with low bacterial count, they can also be more easily identified and confirmed, which helps to prevent missed diagnosis or false negative result (Figures 1E and F).

Among the 3944 cases who underwent both IHC and ^{14}C UBT during gastroscopy (interval < 1 wk, including gastric antrum), the overall positive rate of *H. pylori* infection was 49.7% (1962/3944): 29.8% (1176/3944) of the cases were positive by both ^{14}C UBT and IHC; 2.6% (101/3944) were solely positive for IHC; 17.4% (685/3944) were solely positive for ^{14}C UBT; and 50.3% (1982/3944) were negative. According to the previously established standards, the sensitivity of ^{14}C UBT detection was 94.9% (1861/1962), and the sensitivity of IHC detection was 65.1% (1277/1962). Pearson's χ^2 test confirmed that there was a significant difference between the two methods ($P < 0.001$).

When the *H. pylori* positive rates detected by IHC were compared with the value ranges detected by ^{14}C UBT, it would be 4.2%,11.1%, 25.6%, 52.6%, 66.8% and 73.9% in the value ranges 0-49, 50-100, 101-199, 200-499, 500-999 and ≥ 1000, respectively. For cases negative by ^{14}C UBT, the *H. pylori* positive rate by IHC was 4.8%, while for cases defined as positive by ^{14}C UBT, the *H. pylori* positive rate by IHC was 63.2% (Tables 2 and 3).

To clarify the impact of the difference in the number of biopsies taken during gastric endoscopy on the positive rate by IHC, we divided those cases into two groups: Single biopsy (only 1 specimen) and multiple biopsies (≥ 2 specimens). Pearson's test confirmed that the positive rate for immunohistochemical detection in the single biopsy group was 28.4% (987/3476), and the positive rate in the multiple biopsy group was 40.4% (189/468). The two groups were significantly different ($P < 0.001$) by Pearson χ^2 test, and the *H. pylori*-positive rate in the multiple biopsy group was significantly increased (Table 2).

Correlation between overall infection status of H. pylori and clinical factors such as ethnicity, age, gender and region

According to the *H. pylori* positive criteria, the overall positive rate of *H. pylori* infection was 48.6% (2794/5747). The infection rate in male and female patients was 51.0% (1362/2672) and 46.6% (1432/3075), respectively, with a significant difference by χ^2 test ($P = 0.001$). The *H. pylori* infection rates in the Han and Uyghur ethnic groups were 44.2% (2175/4925) and 75.3% (619/822), respectively, with a significant difference ($P < 0.001$). Further statistical analysis showed that the *H. pylori* infection rate was 47.4% (1091/2303) in Han Chinese males and 41.3% (1084/2622) in Han females, which was a significant difference by χ^2 test ($P < 0.001$). The infection rate was 73.4% (271/369) in Uighur men and 76.8% (348/453) in Uighur women, which was not a significant difference by χ^2 test ($P = 0.291$).

When grouped according to age, the *H. pylori* infection rates in the Han ethnic group were 62.2% (23/37) in the ≤ 30 years group, 45.2% (109/241) in the 31-40 years group, 47.2% (523/1108) in the 41-50 years group, 44.6% (814/1825) in the 51-60 years group, 40.9% (488/1193) in the 61-70 years group, 41.7% (194/465) in the 71-80 years group, and 42.9% (24/56) in the ≥ 81 years group. The *H. pylori* infection rates in the Uyghur ethnic group were 100.0% (16/16) in the ≤ 30 years group, 85.7% (48/56) in the 31-40 years group, 79.2% (171/216) in the 41-50 years group, 76.1% (242/318) in the 51-60 years group, 68.2% (122/179) in the 61-70 years group, 54.1% (20/77) in the 71-80 years group, and data for the ≥ 81 years group were missing. The trend in *H. pylori*-positive rate with age group can be seen in Figure 2.

According to region of residence, the *H. pylori* infection rate was 47.4% (2333/4919) in northern Xinjiang, 66.3% (337/508) in southern Xinjiang, 37.0% (98/265) in the XPCC, and 47.3% (26/55) outside Xinjiang Uyghur Autonomous Region. There was a significant difference in *H. pylori* infection rate among the regions, and the rate in southern Xinjiang was the highest ($P < 0.001$). The *H. pylori*-positive rates of the Han and Uyghur ethnic groups in northern Xinjiang region were 44.5% (1959/4405) and 72.8% (374/514), respectively, and 47.8% (99/207) and 79.1% (238/301) in southern Xinjiang. The infection rates in the Han and Uyghur ethnic groups in the XPCC region were 36.5% (96/263) and 100.0% (2/2), and 42.0% (96/263) and 100.0% (5/5), respectively.

Table 1 The essential epidemic features of cases detected *Helicobacter pylori* with immunohistochemical staining and ^{14}C urea breath test methods (*n* = 5747)

Characteristics		Total	Han	Uyghur
Gender (cases)	Male	2672 (46.5%)	2303 (46.8%)	369 (44.9%)
	Female	3075 (35.5%)	2622 (53.2%)	453 (55.1%)
Age (yr)	Range	19-92	19-92	22-80
	Average	56.3	56.7	53.4
	Median	56.0	56.0	54.0
Residing region (cases)	Northern Xinjiang	4919 (85.6%)	4405 (89.4%)	514 (62.5%)
	Southern Xinjiang	508 (8.8%)	207 (4.2%)	301 (36.6%)
	Xinjiang Production and Construction Corps	265 (4.6%)	263 (5.3%)	2 (0.2%)
	Extra Xinjiang Uyghur Autonomous Region	55 (1.0%)	50 (1.0%)	5 (0.6%)
Total (cases)		5747	4925 (85.7%)	822 (14.3%)

Table 2 Comparison of differences between immunohistochemical staining and ^{14}C urea breath test for detecting *Helicobacter pylori* infection (*n* = 3944)

			H. pylori positive rate (%)					
			Positive[1]	Negative[1]	*P* value[1]	Positive[2]	Negative[2]	*P* value (single *vs* multiple biopsy)[2]
^{14}C UBT (total, *n* = 3944)		Positive	1176 (29.8%)	685 (17.4%)	*P* < 0.001	1962 (49.7%)	1982 (50.3%)	-
		Negative	101 (2.6%)	1982 (50.3%)				
Subgroup	Single biopsy (1 piece, *n* = 3476)	Positive	987 (28.4%)	608 (17.5%)	*P* < 0.001	1068 (30.7%)	2408 (69.3%)	*P* < 0.001
		Negative	81 (2.3%)	1800 (51.8%)				
	Multiple biopsies (≥ 2 pieces, *n* = 468)	Positive	189 (40.4%)	77 (16.5%)	*P* < 0.001	209 (44.7%)	259 (55.3%)	
		Negative	20 (4.3%)	182 (38.9%)				

[1]Immunohistochemical staining.
[2]Immunohistochemical staining & ^{14}C urea breath test combined.
^{14}C UBT: ^{14}C urea breath test; *H. pylori*: *Helicobacter pylori*.

Precise matched comparison for H. pylori infection rates between Han and Uyghur groups

For the 555 matched cases of Uyghur and Han groups, the positive rate of *H. pylori* infection in the Uyghur group was 73.3%, while the positive rate in the matched Han cases was 41.4%. The positive rates of *H. pylori* infection in the Uyghur group were higher than those in the Han group by using IHC (53.2% and 27.7%) or ^{14}C UBT (69.5% and 38.2%). These differences were significant by χ^2 test (all $P < 0.001$). In the matched cases, the infection rate of *H. pylori* in Han men was 47.4%, which was higher than that in Han women (36.6%), which was a significant difference by χ^2 test ($P = 0.012$). The infection rate in Uyghur men was 72.3%, and that of Uyghur women was 74.2%, but there was no significant difference by χ^2 test ($P = 0.630$), Table 4.

Correlation between main disease types diagnosed by gastric biopsy and H. pylori infection

The pathological changes in the H&E-stained slides of gastric mucosa were classified as follows: (1) Benign lesions: 4609 cases of gastritis; 4 with ulcer diagnosed definitively; 440 cases of gastric fundic gland polyps; 502 cases of hyperplastic (regenerative) polyps; three cases of xanthoma; and 36 cases of duodenal ectopic gastric mucosa; (2) Precancerous lesions: Nine cases of LGIN; seven cases of gastric HGIN; two cases of esophageal LGIN; two cases of esophageal HGIN; 92 cases of BE; and one case of duodenal LGIN; and (3) Tumors: 34 cases of gastric adenocarcinoma; four cases of lymphoma; one case of neuroendocrine tumor; and one case of stromal tumor.

To ensure the accuracy of *H. pylori* positive rates, we only show disease types with more than 20 samples here, and the other types are included in Table 5. The *H. pylori* positive rates obtained solely by IHC were as follows: (1) Benign lesions: Gastritis 35.8%, duodenal ectopic gastric mucosa 19.4%, gastric fundic gland polyps 17.3%, and hyperplastic

Table 3 Comparison of the differences between immunohistochemical staining and ^{14}C urea breath test and the in detecting *Helicobacter pylori* infection at different value range levels (*n* = 3944)

Qualitative results[1]	Value ranges (dpm/mmol)[1]	Positive[2]	Negative[2]	Positive ratio (%)[2]	IHC positive rate[2]
Negative (*n* = 2083)	0-49	80	1813	4.2	4.8% (101/2083)
	50-100	21	169	11.1	
Positive (*n* = 1861)	101-199	46	134	25.6	63.2% (1176/1861)
	200-499	193	174	52.6	
	500-999	324	161	66.8	
	≥ 1000	613	216	73.9	

[1]*Helicobacter pylori* of ^{14}C urea breath test.
[2]Immunohistochemical staining for *Helicobacter pylori*.
IHC: Immunohistochemistry.

Table 4 Comparison of *Helicobacter pylori* infection rates between Han and Uyghur undergoing gastroscopy examination among accurately matched pairs (*n* = 555)

Method	*H. pylori* positive rate								
	Male (*n* = 249)[1]	Female (*n* = 306)[1]	*P* value (male/female, Pearson)[1]	Subtotal (*n* = 555)[1]	Male (*n* = 249)[2]	Female (*n* = 306)[2]	*P* value (male/female, Pearson)[2]	Subtotal (*n* = 555)[2]	*P* value (total, Han and Uyghur), Pearson
IHC	89 (35.7%)	65 (21.2%)	*P* < 0.001	154 (27.7%)	134 (53.8%)	161 (52.6%)	*P* = 0.778	295 (53.2%)	*P* < 0.001
^{14}C UBT	111 (44.6%)	101 (33.0%)	*P* = 0.005	212 (38.2%)	170 (68.3%)	216 (70.0%)	*P* = 0.556	386 (69.5%)	*P* < 0.001
IHC & ^{14}C UBT	118 (47.4%)	112 (36.6%)	*P* = 0.012	230 (41.4%)	180 (72.3%)	227 (74.2%)	*P* = 0.630	407 (73.3%)	*P* < 0.001

[1]Han.
[2]Uyghur.
^{14}C UBT: ^{14}C urea breath test; *H. pylori*: *Helicobacter pylori*; IHC: Immunohistochemistry; Pearson: Pearson χ^2 test.

(regenerative) polyps 7.4%; (2) Precancerous lesions: BE 41.3%; and (3) Malignant tumors: 41.2% of gastric adenocarcinoma. If the combination of two detection methods was used, the *H. pylori* infection rate in patients with various types of diseases diagnosed by gastroscopy was higher: (1) Benign lesions: Gastritis 53.8%, gastric fundic gland polyps 27.5%, duodenal ectopic gastric mucosa 22.2%, and hyperplastic (regenerative) polyps 17.5%; (2) Precancerous lesions: *H. pylori*-positive rate of BE was 58.7% (based on gastric samples taken simultaneously, Figure 3); and (3) Malignant tumors: 67.6% gastric adenocarcinoma, Table 5.

Correlation between H. pylori infection rate and grading of chronic or active gastritis, atrophy, and intestinal metaplasia

For gastritis, 91.6% (4222/4609) of the cases showed intact structure of the mucosal lamina propria, which was evaluated and graded according to chronic gastritis, active gastritis, atrophy and intestinal metaplasia. The positive rates for *H. pylori* were as follows: (1) Chronic gastritis: *H. pylori* positive rates of grades 1-3 were 29.9%, 85.2% and 95.6%, respectively; (2) Active gastritis: The positive rates of *H. pylori* in grades 0-3 were 27.6%, 74.9%, 95.6% and 100%, respectively; (3) Atrophy: The positive rates of *H. pylori* in grades 0-3 were 54.4%, 57.3%, 65.5% and 80%, respectively; and (4) Intestinal metaplasia: The positive rates of *H. pylori* in grades 0-3 were 55.6%, 48.6%, 63.5% and 66.7%, respectively. The *H. pylori* infection rates in patients with different grades of chronic gastritis and active gastritis differed significantly (both $P < 0.001$). There was a significant difference in the *H. pylori* infection rate among patients with different grades of intestinal metaplasia, by χ^2 test ($P = 0.032$), indicating that *H. pylori* infection was associated with higher grade of chronic/active gastritis and intestinal metaplasia (Figure 4). However, there was no significant difference ($P = 0.084$ by χ^2 test) in the *H. pylori* infection rates between patients with different grades of atrophy (Table 6).

Table 5 *Helicobacter pylori* infection among main disease types diagnosed by biopsies from endoscopy in Xinjiang Uyghur Autonomous Region (*n* = 5747)

Classification of disease	Pathological diagnosis	*H. pylori* positive rate	
		IHC	IHC & ^{14}C UBT combined
Benign lesions	Gastritis	35.8% (1652/4609)	53.8% (2478/4609)
	Duodenal ectopic gastric mucosa	19.4% (7/36)	22.2% (8/36)
	Gastric fundic gland polyps	17.3% (76/440)	27.5% (121/440)
	Hyperplastic (regenerative) polyps	7.4% (37/502)	17.5% (88/502)
	Ulcer	50.0% (2/4)	100% (4/4)
	Xanthoma	66.7% (2/3)	100% (3/3)
Pre-malignant lesions	Barrett's esophagus	41.3% (38/92)	58.7% (54/92)
	Duodenal LGIN	100% (1/1)	100% (1/1)
	Gastric LGIN	55.6% (5/9)	55.6% (5/9)
	Esophageal HGIN	50.0% (1/2)	100% (2/2)
	Gastric HGIN	28.6% (2/7)	57.1% (4/7)
	Esophageal HGIN	0% (0/2)	0% (0/2)
Malignant tumors	Gastric adenocarcinoma	41.2% (14/34)	67.6% (23/34)
	Lymphoma	75.0% (3/4)	75% (3/4)
	Neuroendocrine tumor	0% (0/1)	0% (0/1)
	GIST	0% (0/1)	0% (0/1)

^{14}C UBT: ^{14}C urea breath test; HGIN: High-grade intraepithelial neoplasia; *H. pylori*: *Helicobacter pylori*; IHC: Immunohistochemistry; LGIN: Low-grade intraepithelial neoplasia; GIST: Gastrointestinal stromal tumor.

DISCUSSION

For diagnosis of *H. pylori* infection, both invasive and noninvasive tests are used. Histology with special stains, rapid urease tests (RUTs), bacterial culture, and polymerase chain reaction (PCR) are examples of invasive tests, which require endoscopy and biopsy. Although PCR-based methods were proposed as gold standard tests[10], expensive, complex, and time-consuming methods, they were not widely used for screening, and were preferred for cases when an etiological role of *H. pylori* was clinically suggested but histopathological confirmation was not possible[21]. To increase the specificity of the histological test, special stains such as modified Giemsa stain, Warthin-Starry Silver stain, and immunohistochemical stain can be used, and IHC is considered to be the most reasonable histological method[21-23]. Serology, stool antigen testing, and UBT are examples of noninvasive tests[24]. UBT is often considered as the gold standard diagnostic test for *H. pylori* infection[10], and consistently produces better results in comparison to many other available tests. IHC and ^{14}C UBT are most widely used in our hospital for routine screening of *H. pylori*. It was not clear whether we needed to perform the two methods simultaneously for *H. pylori* detection, and there were controversial reports about the sensitivity of IHC as being either superior[20] or inferior[19] to ^{14}C UBT. The number of cases of the two studies mentioned above is relatively small, thus the conclusion might have coincidence or selection bias.

The novelty of our study was that it strongly proved that the sensitivity of ^{14}C UBT was significantly higher than that of IHC. We also found about 20% inconsistency between the results of ^{14}C UBT and IHC, although most were negative for IHC and positive for ^{14}C UBT (> 17%). It is speculated that there is a significant focal distribution pattern of *H. pylori* infection in these cases[25,26], and the site and size of biopsy specimen obtained by endoscopic sampling are limited, causing false-negative results[24]. Our research showed that increasing the number of biopsy specimens ($n \geq 2$) significantly improved the positive rate of *H. pylori* detection in pathological IHC methods as expected, and the greater the number of biopsy specimens, the fewer false-negative results from sampling errors[24]. However, it might be unacceptable to do multiple biopsies for almost-healthy patients. ^{14}C UBT can overcome this drawback and reflect the overall *H. pylori* infection status in the upper gastrointestinal tract[18].

We should point out that ^{14}C UBT might be unreliable when the reported value is around the cut-off value, and these cases might be infected with a lower density of *H. pylori*[10,27]. That could explain why a small number of cases (< 3%) were negative for ^{14}C UBT but positive for IHC in our study. IHC is superior for tracing *H. pylori* infections in such circumstances, relying on both morphological review and the specificity of the *H. pylori* antibodies used for IHC[15]. The specificity of the primary antibody used in IHC prevents mistaking other resident or contaminating bacteria for *H. pylori* [13,15].

Table 6 Correlation between grading (chronic/active, atrophy, intestinal metaplasia grade) and *Helicobacter pylori* infection rate in gastritis

Characteristics	Grading	*H. pylori* positive rate		
		IHC	IHC & ^{14}C UBT combined[1]	*P* value
Chronic	G1	13.3% (312/2348)	29.9% (703/2348)	$P < 0.001$[2]
	G2	65.6% (1110/1692)	85.2% (1441/1692)	
	G3	81.3% (148/182)	95.6% (174/182)	
Active	G0	10.5% (229/2174)	27.6% (599/2174)	$P < 0.001$[2]
	G1	54.1% (626/1157)	74.9% (867/1157)	
	G2	80.3% (705/878)	95.6% (839/878)	
	G3	76.9% (10/13)	100% (13/13)	
Atrophy	G0	37.0% (1367/3697)	54.4% (2010/3697)	$P = 0.084$[1]
	G1	37.2% (170/457)	57.3% (262/457)	
	G2	46.6% (27/58)	65.5% (38/58)	
	G3	60.0% (6/10)	80.0% (8/10)	
Intestinal metaplasia	G0	38.8% (1235/3183)	55.6% (1771/3183)	$P = 0.032$[1]
	G1	30.9% (289/934)	48.6% (480/934)	
	G2	43.8% (42/96)	63.5% (61/96)	
	G3	44.4% (4/9)	66.7% (6/9)	

[1]Fisher's exact probability method.
[2]Pearson χ^2 test.
^{14}C UBT: ^{14}C urea breath test; G0: Grade 0 = none; G1: Grade1 = mild; G2: Grade 2 = moderate; G3: Grade 3 = severe; *H. pylori*: *Helicobacter pylori*; IHC: Immunohistochemistry.

We showed that although IHC gave direct evidence of *H. pylori* infection, its sensitivity was lower and there was a higher risk of false-negative results. ^{14}C UBT is still the preferred method for detecting *H. pylori* infection clinically. However, to improve the positive rate of *H. pylori* detection, we recommend performing ^{14}C UBT and IHC simultaneously, especially for patients unexplained gastritis, previously treated with low organism density, and with results around the cut-off value[15]. Our statistical analysis supports that the *H. pylori* positive rate can increase significantly as multiple endoscopic biopsies are submitted. One limitation of our was is that we did not perform a PCR-based method as the gold standard. However, potential pitfalls still exist with PCR methods, and more studies need to be conducted on diagnostic tests for *H. pylori* to find a reliable gold standard. Positive findings with either IHC or ^{14}C UBT are highly specific[14,28], and are widely recognized to establish diagnosis of *H. pylori* infection[18,29,30]; thus, we focused on their sensitivity.

H. pylori infection is found worldwide, but the rate varies significantly among different countries (11%-91%)[29]. China has a high incidence of *H. pylori* infection, and it has decreased to 40%-60% after economic development and changes in lifestyle[30,31]. Our study showed that the overall infection rate of *H. pylori* in a single center in Xinjiang Uyghur Autonomous Region (Urumqi) was 48.6%, which was close to the national infection rate[30,31]. However, the infection rate was significantly higher than previously reported (36.5%) in Shihezi (located adjacent to Urumqi) in the XPCC[7]. We speculate that *H. pylori* infection was detected by morphological examination without special stains, and was lower in sensitivity. The majority of the population in Shihezi area is of Han nationality. The overall infection rate of *H. pylori* in this center was also higher than the 43.6% overall positive infection rate reported by the First Affiliated Hospital of Xinjiang Medical University in the same region of Urumqi in 2012[6], which used a combination of RUT and ^{14}C UBT. Thus, the higher *H. pylori* positivity rate in our center was likely related to the higher sensitivity of our immunohistochemical assay compared to their RUT assay. Our overall infection rate was lower than that reported in Yili region[9], because the majority of cases in the other study were from multiple ethnic groups, whose *H. pylori* infection rate were all higher than in the Han group.

Xinjiang Uyghur Autonomous Region is a vast region with multiple ethnic groups living together. Previous reports have shown that there are differences in *H. pylori* infection rates between different genders, ages, ethnic groups, and regions[6-9]. Our single center data also showed that the overall infection rate in males was significantly higher than in females, which is consistent with reports in the Shihezi area[7]. However, these were inconsistent with the reports in the Ili area[9], with no significant gender difference. We believe that both our cases and the cases in Shihezi area were randomly enrolled with a large sample size, and the majority were Han ethnicity. The infection rate among Uyghur women in our study was also higher than in men, and the proportion of ethnic minorities in the study from Ili region[9] is

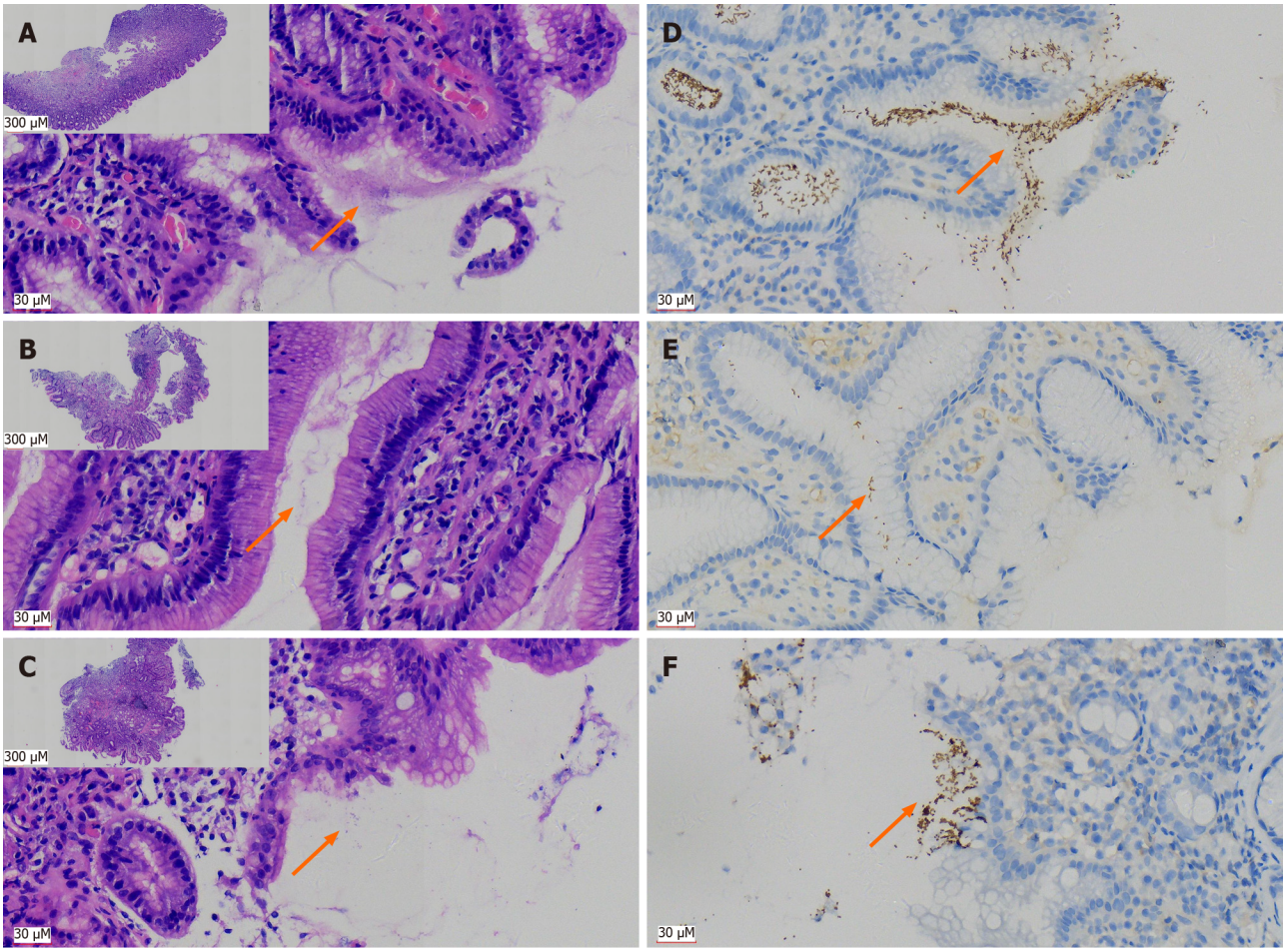

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Figure 1 Immunohistochemical staining using antibodies against *Helicobacter pylori* has significant advantages over hematoxylin and eosin staining in the pathological tissue for gastroscopy biopsy. A: Distribution of curved rod-shaped bacteria [hematoxylin and eosin (H&E, 400 ×)], on the surface of glands and in the mucus of typical *Helicobacter pylori* (*H. pylori*)-positive patients in H&E section; B: A case with low bacterial count of *H. pylori* (H&E, 400 ×); C: A case with atypical *H. pylori* morphology (mainly coccoid rather than rod-shaped), which was difficult to identify (H&E, 400 ×), lower magnification figures of A-C are in the upper left corner (H&E, 40 ×); D: Immunohistochemistry (IHC) with antibodies against *H. pylori* indicated a large number of bacteria, exhibiting brown yellow deep staining (DAB, high magnification, one case the same as in A); E: IHC displayed *H. pylori* bacilli more clearly than H&E due to a lower bacterial count (DAB staining, high magnification, one case the same as in B); F: Atypical coccoid *H. pylori* were clearly present and confirmed by IHC (DAB staining, high magnification, one case the same as in C); orange arrows in A-F indicate distribution of *H. pylori*.

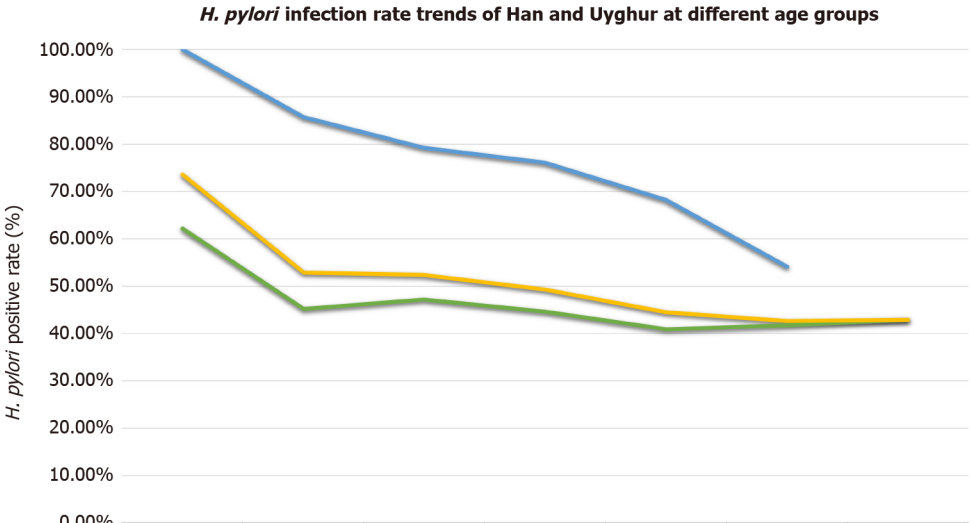
larger than in our cases from Urumqi.

From a perspective of regional difference, patients from southern Xinjiang had the highest positive rate of *H. pylori* infection, followed by patients from northern Xinjiang, and patients from the XPCC had the lowest *H. pylori* infection rate. We speculate that it may be related to the fact that southern Xinjiang is mainly inhabited by Uyghur ethnic groups, while northern Xinjiang is mainly inhabited by Han ethnic groups, as is the XPCC[7].

These results mentioned above suggest that ethnic difference is the most important cause of difference in *H. pylori* infection rate, which was confirmed by our matched comparative analysis. The differences in ethnicity might be one of the most important factors affecting the epidemic characteristics of *H. pylori* infection. The positive rate for *H. pylori* obtained by combining the two most sensitive methods in Uyghur was 75.3%, which was higher than previously reported (59.8%-62.38%)[6,8,9], indicating that the prevention and control for *H. pylori* infection in Uyghur people might be more challenging.

In addition, regardless of the Uyghur or Han ethnic groups, the *H. pylori* infection rate calculated by age group showed a decreasing trend as age increased. In the Han ethnic group, there was an increase after the age of 70 years, which is consistent with previous reports[6,32,33], indicating that we might need to eradicate *H. pylori* infection in young people as early as possible.

Since our hospital is a general hospital focused on traditional Chinese medicine, the majority of patients have mild stomach discomfort. Overall, benign inflammatory lesions were the main cause (> 90%) in this continuous randomized study. Therefore, there were few cases of malignant lesions such as gastric adenocarcinoma included in this study. Malignant tumors have a closer correlation with *H. pylori* infection, with over two-thirds of gastric adenocarcinoma cases and 75% of lymphoma cases having *H. pylori* infection. According to the literature, eradicating *H. pylori* in the first-degree relatives of gastric cancer patients is an important factor in reducing the incidence of gastric cancer[34]. In addition, *H. pylori*-related gastric lymphoma patients can achieve complete remission by eradicating *H. pylori*[35,36]. BE is a precan-



	≤ 30	31-40	41-50	51-60	61-70	71-80	≥ 81
Han	62.20%	45.20%	47.20%	44.60%	40.90%	41.70%	42.90%
Uyghur	100.00%	85.70%	79.20%	76.10%	68.20%	54.10%	
Total	73.60%	52.90%	52.40%	49.30%	44.50%	42.60%	42.90%

DOI: 10.3748/wjg.v29.i43.5834 **Copyright** ©The Author(s) 2023.

Figure 2 *Helicobacter pylori* positive rate changed with age in Xinjiang Uyghur Autonomous Region. Regardless of the Uyghur, Han or the overall series, the *Helicobacter pylori* (*H. pylori*) infection rates calculated by age group showed a decreasing trend as age increased. In Han group, the positive rate of *H. pylori* infection increased in two age groups after the age of 70 years. *H. pylori*: *Helicobacter pylori*.

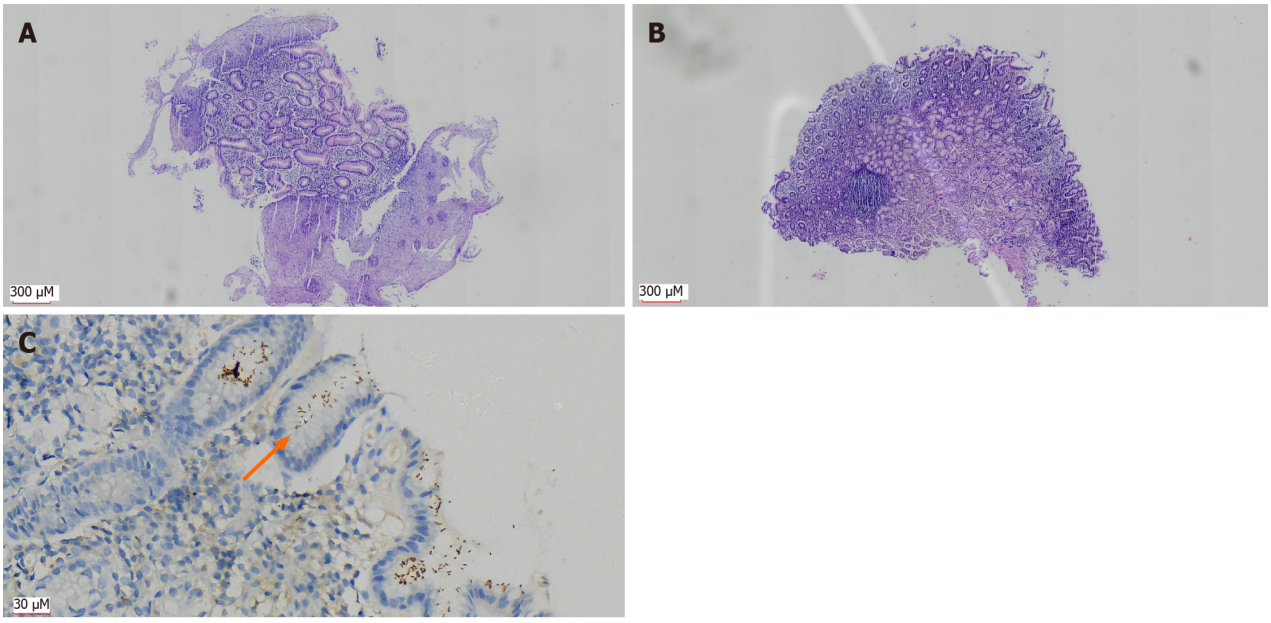


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Figure 3 Patients with Barrett's esophagus concurrent with *Helicobacter pylori* infection. A: Changes with Barrett's esophagus: Many hyperplastic glands in the esophageal wall, partially replacing the squamous epithelial mucosa [hematoxylin and eosin (H&E, 40 ×)]; B: Mild inflammation (H&E, 40 ×) in the gastric wall tissue biopsied simultaneously; C: Immunohistochemistry showing *Helicobacter pylori* (*H. pylori*) infection (DAB, 400 ×); orange arrow in C indicates distribution of *H. pylori*.

cerous lesion and some studies have shown that *H. pylori* infection and BE are inversely related[37,38]. The *H. pylori* infection rate (58.7%) was higher than the overall positive level, and BE may be related to *H. pylori* infection; however, the positive association between *H. pylori* infection and BE seemed to be a paradox. Well-designed prospective cohort studies with a powered sample size are required[39].

For gastritis, the *H. pylori* infection rate significantly increased with grade of chronic/active gastritis, and intestinal metaplasia grading. Infection with *H. pylori* may be an important factor leading to gastritis and intestinal metaplasia[40]. Eradicating *H. pylori* can improve the inflammatory response of the gastric mucosa, prevent or delay development of atrophy and intestinal metaplasia[40,41], block the Correa mode of intestinal gastric cancer evolution, and eliminate the

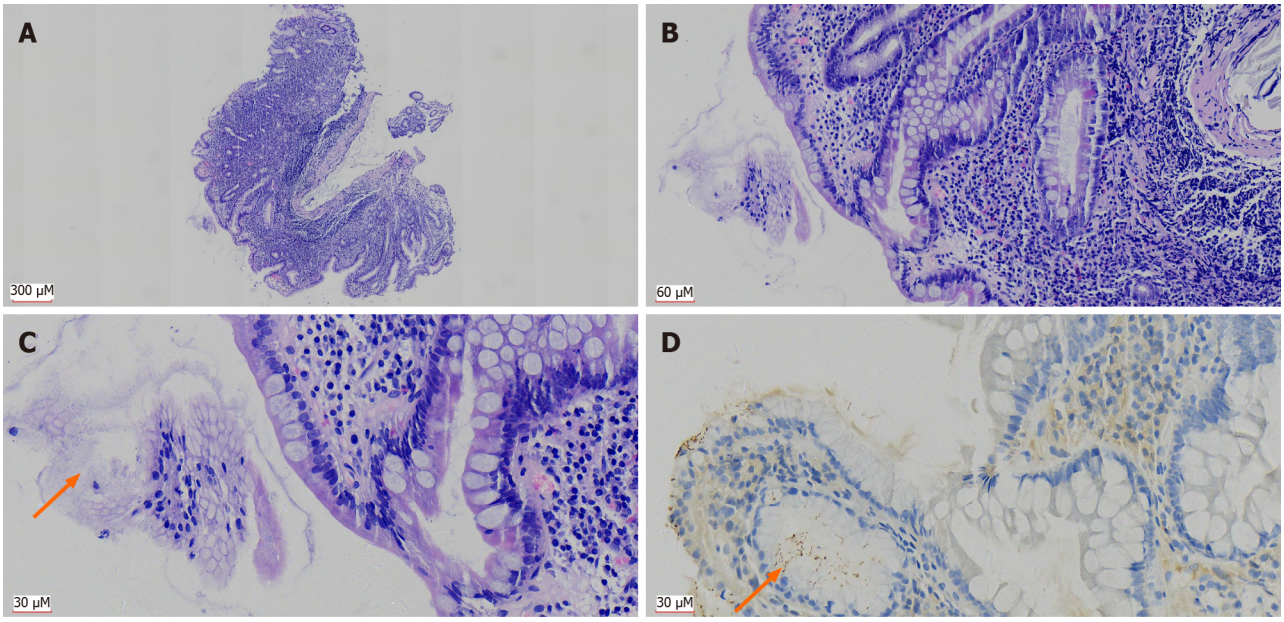

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Figure 4 *Helicobacter pylori* infection associated with elevated grade of chronic/active gastritis and intestinal metaplasia. A: Patient with severe chronic gastritis (grade 3) in the gastric wall biopsy tissue; inflammatory cell infiltration can be seen throughout the lamina propria of the mucosa [hematoxylin and eosin (H&E, 40 ×)]; B: Infiltrating inflammatory cells were mainly lymphocytes, with scattered neutrophils, indicating chronic gastritis combined with active gastritis (H&E, 200 ×); C: Gastric glands were infected by *Helicobacter pylori* (*H. pylori*) and were accompanied by severe intestinal metaplasia (H&E, 400 ×); D: *H. pylori* infection was confirmed by immunohistochemistry (DAB, 400 ×); all images were from the same patient, orange arrows in C and D indicates distribution of *H. pylori*.

risk of (intestinal) gastric cancer[18,29,33,34]. By combining the IHC and ^{14}C UBT results, we can obtain a better linear correlation between *H. pylori* infection and severity of gastritis, thus it is important to use two diagnostic tests at the same time.

CONCLUSION

Our results show that the sensitivity of ^{14}C UBT for detecting *H. pylori* is significantly higher than that of IHC with pathological specimens obtained by gastroscopy. The combination of ^{14}C UBT and IHC is necessary to improve the detection rate of *H. pylori* infection, and increasing the number of biopsy specimen (≥ 2) can significantly improve the positive rate of *H. pylori* detection in pathological immunohistochemical methods. The overall *H. pylori* infection rate in Xinjiang Uyghur Autonomous Region was higher than previously reported, and ethnicity was the most important factor for detection by combination of the two methods. Uyghur people have a higher *H. pylori* infection rate. The overall *H. pylori* infection rate calculated by age group showed a decreasing trend as age increased. Therefore, the key populations for the prevention and control of *H. pylori* infection in Xinjiang Uyghur Autonomous Region are the Uyghur and young people.

ARTICLE HIGHLIGHTS

Research background

There are differences in *Helicobacter pylori* (*H. pylori*) infection rate in Uyghur and Han ethnic groups. ^{14}C urea breath test (^{14}C UBT) and immunohistochemistry (IHC) with tissue from gastroscopic biopsy are widely used detection methods, but both lack large cohort studies to accurately evaluate their performance.

Research motivation

To compare the difference between ^{14}C UBT and IHC for accurate testing for *H. pylori* infection, and to study the difference in infection positive rate between Uyghur and Han ethnic groups.

Research objectives

We included 5747 cases with *H. pylori* infection detected by both IHC and ^{14}C UBT. We detected 3944 by simultaneous IHC and ^{14}C UBT and 555 pairs of Han/Uyghur were compared for their *H. pylori* infection rate.

Research methods

IHC and ^{14}C UBT were performed at the same time (interval < 1 wk, with sampling site including gastric antrum), and 3944 cases were screened out. The overall *H. pylori* infection positive rate was calculated by combining IHC and ^{14}C UBT results (n = 5747). Correlation between *H. pylori* infection and patients' clinical parameters (gender, age, ethnicity and region) was analyzed. 555 pairs of Han/Uyghur cases (completely matched for gender and age) were compared for their *H. pylori* infection rates. The *H. pylori* infection rate and pathological diagnosis, including gastritis (chronic/active inflammation, atrophy, and intestinal metaplasia), were analyzed.

Research results

Among the 3944 cases for which ^{14}C UBT and IHC were performed at the same time, the sensitivity was 94.9% for ^{14}C UBT and 65.1% for IHC, which was a significant difference (P < 0.001). Among those positive by ^{14}C UBT (detection value > 100), the *H. pylori* positive rate with IHC was 63.2%, and among those negative for ^{14}C UBT (detection value ≤ 100), the IHC positive rate was 4.8%. In combination with both detection methods, the total rate of *H. pylori* infection in all 5747 patients was 48.6%, and there were significant differences for gender, age, ethnicity, and region (P values were 0.001, < 0.001, < 0.001 and < 0.001). The *H. pylori* infection rates for the 555 Chinese/Uyghur paired cases (completely matched for gender and age) were 41.4% and 73.3%, which was a significant difference (P < 0.001). For benign gastric lesions, the combined *H. pylori* infection rate was 53.8% for inflammation, 27.5% for fundus gland polyps, 22.2% for duodenal ectopic gastric mucosa, 17.5% for hyperplastic polyps, 58.7% for BE, and 67.6% for gastric adenocarcinoma. Positivity for *H. pylori* infection was significantly related to moderate-severe (grade 2-3) chronic inflammation, moderate-severe active inflammation and moderate-severe (grade 2-3) intestinal metaplasia (P < 0.001, < 0.001 and 0.032 in order).

Research conclusions

The sensitivity of ^{14}C UBT was significantly higher than that of IHC when detecting *H. pylori* infection, but there were still *H. pylori* positive cases missed that were detected by IHC. Combination of both methods can increase the detection accuracy of *H. pylori* infection, and the overall infection rate of *H. pylori* in our study was higher than previously reported in Xinjiang Uyghur Autonomous Region. Ethnic difference was the most important factor affecting the *H. pylori* infection rate, and the Uyghur people had more *H. pylori* infection. The *H. pylori* infection rate decreased with age, and was more correlated with precancerous lesions and malignant tumors, and increased with severity of inflammation.

Research perspectives

Our study highlights the importance of using IHC and ^{14}C UBT together for *H. pylori* infection, and the prevention and intervention of *H. pylori* infection in Xinjiang Uyghur Autonomous Region and emphasizes that the Uyghur and young people should be examined as early as possible.

FOOTNOTES

Co-first authors: Yu-Hua Peng and Xue Feng.

Author contributions: Peng YH and Feng X collected samples and clinicopathological data, participated in the data analysis and drafted the manuscript; Zhou Z finished the pathological experimental procedures; Yang L performed the statistical analysis; Shi YF designed and supervised the study, revised the manuscript; and all authors read and approved the final manuscript.

Institutional review board statement: The study was approved by the Science and Research Office of Traditional Chinese Medical Hospital of Xinjiang Medical University (Urumqi).

Informed consent statement: As a pure retrospective study, and no intervention and impacts on the diagnosis and treatment of the patients involved, informed consent in our study was waived as approved by the Ethics Committee of the hospital.

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ORCID number: Yu-Hua Peng 0009-0002-1297-063X; Xue Feng 0009-0005-6672-1913; Zhong Zhou 0009-0007-8179-8147; Lei Yang 0000-0002-9514-3789; Yun-Fei Shi 0000-0002-0417-5727.

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ORIGINAL ARTICLE

Basic Study

Chemical components and protective effects of *Atractylodes japonica* Koidz. ex Kitam against acetic acid-induced gastric ulcer in rats

Bi-Xian Zhen, Qian Cai, Feng Li

Specialty type: Gastroenterology and hepatology

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Bi-Xian Zhen, Qian Cai, Feng Li, Department of Medicine, Liaoning University of Traditional Chinese Medicine, Dalian 116600, Liaoning Province, China

Corresponding author: Feng Li, PhD, Professor, Department of Medicine, Liaoning University of Traditional Chinese Medicine, No. 77 Shengming 1st Road, Double D Port, Dalian 116600, Liaoning Province, China. zhanglijiayi@163.com

Abstract

BACKGROUND

Atractylodes japonica Koidz. ex Kitam. (*A. japonica*, Chinese name: Guan-Cangzhu, Japanese name: Byaku-jutsu), a perennial herb, which is mainly distributed in northeast area of China, it's often used to treat digestive system diseases such as gastric ulcer (GU). However, the mechanism of its potential protective effects against GU remains unclear.

AIM

To investigate the protective effects of *A. japonica* on acetic acid-induced GU rats.

METHODS

The chemical constituents of *A. japonica* were determined by ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) analysis. The rat model of GU was simulated by acetic acid method. The pathological changes of gastric tissues were evaluated by hematoxylin-eosin stain, the levels of epidermal growth factor (EGF), EGF receptor (EGFR), nuclear factor kappa-B (NF-κB), interleukin-1β (IL-1β), IL-10, Na^+-K^+-ATPase (NKA) in serum and gastric tissues were determined by enzyme-linked immunosorbent assay, and the mRNA expressions of EGFR, NF-κBp65, IkappaBalpha (IκBα) and Zonula Occludens-1 (ZO-1) in gastric tissues were determined by real-time reverse transcription polymerase chain reaction, and the efficacy was observed. Then, plasma metabolomic analysis was performed by UPLC-MS/MS to screen the specific potential biomarkers, metabolic pathways and to explore the possible mechani-sms.

RESULTS

48 chemical constituents were identified. Many of them have strong pharmacological activity, the results also revealed that *A. japonica* significantly improved the pathological damage of gastric tissues, increased the expression levels of IL-10,

IκBα related to anti-inflammatory factors, decreased the expression levels of IL-1β, NF-κB, NF-κBp65, related to proinflammatory factors, restored the levels of factors about EGF, EGFR, ZO-1 associated with ulcer healing and the levels of factors about NKA associated with energy metabolism. Metabolomic analysis identified 10 potential differential metabolites and enriched 7 related metabolic pathways.

CONCLUSION

These findings contribute to the understanding of the potential mechanism of *A. japonica* to improve acetic acid-induced GU, and will be of great importance for the development and clinical application of natural drugs related to *A. japonica.*

Key Words: *Atractylodes japonica* Koidz. ex Kitam.; Ulcer; Acetic acid; Digestive system diseases; Metabolomics; Rats

Core Tip: *Atractylodes japonica* Koidz. ex Kitam. is a commonly used folk medicine for the treatment of gastric ulcer (GU), but the mechanism of the treatment of GU is still unclear. In this study, chemical composition, pharmacodynamics and metabolomics were studied to explore the potential mechanism of it. The finding was closely related to anti-inflammation, ulcer healing and other factors.

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INTRODUCTION

Gastric ulcer (GU) is a common global gastrointestinal disorder with common symptoms such as loss of appetite, stomach pain, acid reflux, gastric distention, nausea, and in severe cases, gastric bleeding[1]. Due to its long treatment cycle, relapsing easily, and other characteristics, it has serious physiological and psychological effects on patients. Although the mechanism of ulceration is not clear, pathological studies have found that gastric mucosal lesions are significantly contributed to the development of ulcers[2]. With the accelerated pace of life and bad health habits such as excessive alcohol consumption, smoking, use of non-steroidal anti-inflammatory drugs[3], and increased psychological burden from work, study, family and social environment , the incidence of GU has been rising every year. Therefore, the treatment of GU has become one of the hotspots in research[4]. Although current treatments with traditional western drugs such as proton-pump inhibitors, H2-receptor antagonists, M1-receptor antagonists, and antibiotics against *Helicobacter pylori* are effective, their side effects are often unavoidable[5-7]. Therefore, it is imperative to explore new therapeutic agents. However, herbal medicines for GU have a long history and have received considerable attention for their high efficacy, low side effects, and affordability[8].

Atractylodes japonica (*A. japonica*) is a perennial herb in the Asteraceae family that grows on hillsides, bushes, tussah forests, *etc* The herb has irregular masses or irregularly curved cylinders (3-8 cm in length, 2-3cm in diameter)[9] and is mainly distributed in the Siberian region of Russia, northeastern China, Korea, and Japan[10]. The earliest record of *Atractylodes* in ancient books can be found in Shennong's Classic of Materia Medica[11]. In the 2020th edition of the Chinese Pharmacopoeia, *Atractylodes lancea* (Thunb.) DC. and *Atractylodes chinensis* (DC.) Koidz. were included as sources of *Atractylodis rhizoma*, *A. japonica* was not included in it[12], but was included in the Japanese Pharmacopoeia as a source of *Atractylodis rhizoma*. The dried rhizome of *A. japonica* is commonly used in the treatment of various diseases, such as loss of appetite, indigestion, abdominal distension, diarrhea, night blindness, rheumatic arthritis, *etc*[13]. The chemical components of *A. japonica* involve sesquiterpenoids, acetylene, saccharides, *etc*[14]. Modern pharmacological studies have shown that extracts of *A. japonica* have a variety of biological activities, including anti-inflammation, anti-viral, hypoglycemic, diuretic, cardioprotective, and other bioactive effects, along with specific efficacy in the treatment of digestive disorders such as GU[10,15,16]. In recent years, the demand for *A. japonica* at home and abroad has gradually increased.

There are a few studies on *A. japonica's* chemical composition, which forms the basis for its mechanism of action. Although *A. japonica* has a good therapeutic effect, there is little information about its anti-ulcer activity and related mechanisms. In the preliminary study, the research group who adopted the rheumatoid arthritis model established by Freund's complete adjuvant method, which also proved to have a better anti-inflammatory effect[17]. Therefore, this study intends to focus on the potential anti-inflammatory and protective effects of *A. japonica*. In addition, because of its comprehensive and dynamic characteristics, metabolomics is often used in the study of diseases. According to the changes in endogenous metabolites in the body after the intervention of herbal medicines, the mechanism of action is systematically explained. In this study, pharmacodynamic and metabolomic approaches were used to elucidate the protective mechanism of *A. japonica* in a model for acetic acid-induced GU in rats. Thus, our study may provide a

theoretical basis for the application of *A. japonica.*

MATERIALS AND METHODS

Plant materials and reagents

Collected *Atractylodes japonica* Koidz. ex Kitam (Kuandian, Liaoning, China) was identified as the rhizome of *A. japonica* by Professor Feng Li and deposited in Liaoning University of Traditional Chinese Medicine. Omeprazole was purchased from Shiyao Group Ouyi Pharmaceutical Co., Ltd. (Shijiazhuang, Hebei, China) (Drug approval number: H20046430). This drug is commonly used in clinical treatment of GU and as a positive drug for experimental studies[18,19], transformed into powder, and prepared into a 2 mg/mL solution before use. The drug was crushed through a 180-mesh sieve. Low, middle, and high doses of *A. japonica* were prepared into suspensions of 0.047 g/mL, 0.094 g/mL and 0.188 g/mL, respectively, and shaken well before use. Formic acid, methanol, and acetonitrile (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NJ, United States). The SweScript RT I First Strand cDNA Synthesis Kit was provided by Servicebio (Wuhan, Hubei, China). Yeasen Biotechnology (Shanghai, China) provided HieffTM qPCR SYBR® Green Master Mix (No Rox Plus). Enzyme-linked immunosorbent assay (ELISA) kits of epidermal growth factor (EGF), EGF receptor (EGFR), nuclear factor kappa-B (NF-κB), interleukin-1β (IL-1β), IL-10, and Na^+-K^+-ATPase (NKA) were purchased from Lianshuo Biotechnology Co., Ltd. (Shanghai, China).

Sample preparation and detection

Approximately 1 g of *A. japonica* (through 60 mesh sieve) was added to 20 mL of 80% methanol (v/v) for 40 min using ultrasonication, centrifuged for 10 min at 13780 × *g* and the supernatants were obtained. Finally, the supernatants were filtered through a 0.22 μm membrane filter. Then, the sample was analyzed using LC-MS (UPLC, Thermo, United States, Q Exactive MS, Thermo, United States) platform. Chromatographic analysis was performed using a ACQUITY UPLC C18 Column (100 mm × 2.1 mm, 1.8 μm, Waters, United States). The mobile phases consisted of acetonitrile (A) and 0.1% aqueous formic acid (v/v) (B). The analysis was carried out with an elution gradient using the following steps: 0-5 min, 95%-46% B; 5-11 min, 46% B; 11-20 min, 46%-23% B; 20-30 min, 23%-5% B, with the flow rate at 0.3 mL/min. The column temperature was kept at 30 °C, and the injection volume was 2 μL. The ESI source conditions were set as follows: Sheath gas flow rate as 35 Arb, aux gas flow rate as 15 Arb, capillary temperature as 320 °C, full MS resolution as 70000, MS/MS resolution as 17500, collision energy as 40 in NCE mode, and spray voltage as 4.0 kV (positive) or -3.0 kV (negative), respectively. The mass range scanned was 100-1500 m/z. Furthermore, MS data were collected with Xcalibur software (version 3.0).

Animals and treatment

Male Sprague-Dawley rats (specific pathogen-free grade), weighing 180-220 g, were purchased from Liaoning Changsheng Biotechnology Co., Ltd. (SCXK 2020-0001, Liaoning, China). Prior to the experiment, the rats were raised under normal laboratory conditions: Housed for 7 d at 24 °C and 45%-55% relative humidity. After acclimatization for 7 d, all rats were randomly divided into the normal group (NG), the acetic acid-induced model group (MG), the omeprazole group (OG), the low dose group of *A. japonica* (LA), the middle dose group of *A. japonica* (MA), and the high dose group of *A. japonica* (HA), n = 8 for each group. GU was induced by acetic acid treatment in rats according to the method of Okabe *et al*[20] and Okabe *et al*[21] with partial modifications[19]. All rats were fasted for 24 h, and then a laparotomy was performed through a left subcostal incision after anesthesia with isoflurane. The stomach was gently exteriorized and clamped 3 mm away from the pylorus with special tweezers, a double-ring tweezer with a 9-mm diameter that can clamp the injection site[19]. Furthermore, a 0.2 mL mixture of mineral oil and 60% acetic acid (v/v) in the same syringe was injected into the subserosal layer in the glandular portion of the anterior wall in the clamping region, and the solution was aspirated off after 45 s. In the NG group, saline was used instead of acetic acid. The opened abdomen was then cautiously placed back, cleaned with penicillin, and sutured. Then the rats were fed normally. Except for the NG and MG groups, they were given the same amount of normal saline for 10 d (20 mL/kg/d), twice a day. Other groups were treated with intragastric administration for 10 d (20 mL/kg/d), twice a day. 12 h after the last administration, all rats were anesthetized[22]. Blood was collected from rats, then centrifuged at 3910 × *g* for 10 min. The clear serum and plasma for ELISA assays and metabolomics were then stored at -80 °C until the measurement. The stomach was removed and washed with ice-cold saline. The gastric mucosal injury was observed and evaluated by calculating ulcer score[23]. Then, the ulcer tissue was cut into three parts, and one part was immediately fixed with a 4% paraformaldehyde solution for pathological analysis. Other parts were used for ELISA and mRNA level assays; they were also stored at -80 °C until the measurement.

Gastric histopathology examination

Gastric tissues were immediately fixed with a 4% paraformaldehyde solution for pathological analysis; gradient alcohol and xylene were used for dehydration and transparency separately. The tissues were impregnated with wax and embedded. The sections were made to a thickness of 5 μm, stained with hematoxylin for 30 min, counterstained with eosin, dehydrated with gradient alcohol, thoroughly permeabilized with xylene, sealed with neutral resin, observed under a light microscope (BX53, Olympus, Tokyo, Japan), and blind analysis was performed by an experienced histopathologist.

ELISA analysis

Serum and gastric tissues were thawed at 4 °C before use. Gastric tissues, weighing 100 mg, were homogenized into a 10% tissue homogenate prepared by adding 900 μL of ice-cold saline, centrifuging for 10 min at 3910 × *g*, and obtaining the supernatants. The levels of EGF, EGFR, NF-κB, IL-1β, IL-10, and NKA were analyzed by ELISA (Infinite M200, TECAN, Switzerland) at 450 nm.

Gene expression analysis

According to the supplier's instructions, the trizol method was used to extract total RNA[24], 2.5 μL of the solution to be tested was aspirated, and RNA concentration and purity were detected using an ultra-micro spectrophotometer (Nanodrop 2000, Thermo, America). The SweScript RT I First Strand cDNA Synthesis Kit was used to reverse transcribe to cDNA; the specific reaction system was 20 μL. The HieffTM qPCR SYBR® Green Master Mix was used to perform DNA amplification. Primers were provided by Tianyi Huiyuan Biotechnology Co., Ltd. (Wuhan, China), and data were analyzed using the comparative cycle threshold (CT) method. The primers for the gene sequences are shown in Table 1.

Metabolomics analysis

Plasma samples were thawed on ice. 100 μL sample was taken and placed into an EP tube, extracted with 300 μL methanol, vortexed for 30 s, and then ultrasound treated for 30 min (incubated in ice water) and incubated for 1 h at -40 °C to precipitate proteins, followed by vortexing for 30 s, and incubated for 0.5 h at 4 °C. Then the sample was centrifuged at 13780 × *g* for 15 min at 4 °C. The entire supernatant was collected and placed into an EP tube and incubated for 1 h at -40 °C after centrifugation at 13780 × *g* for 15 min at 4 °C. 200 μL of supernatants, including 5 μL internal standard (2-chloro-DL-phenylalanine, 0.5 mg/mL), were transferred to LC-MS vials by mixing. The quality control (QC) samples were prepared by mixing an equal aliquot of the supernatants from all samples. Plasma samples were analyzed by LC-MS (UPLC, Waters, United States; Q Exactive MS, Thermo, United States). Chromatographic analysis was performed using an UPLC HSS T3 column (2.1 mm × 100 mm, 1.8 μm, Waters, United States). The mobile phase consisted of 0.05% formic acid in water (A) and acetonitrile (B). The analysis was carried out with an elution gradient using the following steps: 0-1 min, 5% B; 1-12 min, 5%-95% B; 12-13.5 min, 95% B; 13.5-13.6 min, 95%-5% B; 13.6-16 min, 5% B, with the flow rate of 0.3 mL/min. The column temperature was kept at 40 °C. The autosampler temperature was 4 °C, and the injection volume was 5 μL. The ESI source conditions were set as follows: Sheath gas flow rate as 45 Arb, aux gas flow rate as 15 Arb, capillary temperature as 350 °C, full MS resolution as 70000, MS/MS resolution as 17500, collision energy as 15/30/45 in NCE mode, and spray voltage as 3.0 kV (positive) or -3.2 kV (negative), respectively.

Data process and multivariate analysis

First of all, the sample data were obtained with feature extraction and preprocessed with Compound Discoverer software (version 2.1, Thermo, United States). Then, the normalized data were imported into SIMCA-P software (version 14.1, Umetrics, Umea, Sweden) for performing multivariate statistical analysis (MVA), including principal component analysis (PCA) and orthogonal projections to latent structures-discriminant analysis (OPLS-DA)[25]. The validity of the OPLS-DA model was evaluated based on the results of the permutation test. Meanwhile, METLIN (http://metlin.scripps.edu/), HMDB (http://www.hmdb.ca/), and KEGG (http://www.kegg.com/) were used to identify potential biomarkers by comparing the mass spectrometry fragmentation information. Furthermore, potential markers were visualized in the enrichment pathway using MetaboAnalyst (http://www.metaboanalyst.ca/).

Statistics analysis

The results were expressed as the mean ± SD. Student's *t*-test, one-way analysis of variance (ANOVA) by Dunnett's post-hoc test and Kruskal-Wallis test were used for statistical analysis. $P < 0.05$ was statistically significant, and $P < 0.01$ was highly significant. GraphPad Prism software (version 9.5, San Diego, United States) and TBtools (version 1.108, Guangzhou, China) were used for visualization.

RESULTS

Identification of the constituents by UPLC Q-Exactive Orbitrap MS

UPLC Q-Exactive Orbitrap was employed for comprehensive analysis in positive and negative modes to identify the chemical constituents using related databases and literature. Therefore, a total of 48 components (Table 2) were identified as terpenoids, flavonoids, organic acids, *etc.* A typical total ion chromatogram of *A. japonica.* in positive and negative ion modes are shown in Figure 1.

Effects of A. japonica on GU by histological analysis

Macroscopic analyses showed the gastric mucosa in NG group was smooth and complete with continuous mucosal plica and no edema. The gastric mucosa of MG group showed swelling and congestion. The treatment group and the positive drug group had different degrees of improvement, the surface of gastric mucosa in OG group was more flat, with no obvious ulcer surface. The ulcer was round or nearly round in the treated group, with slight bulge around the ulcer, and the ulcer area was smaller than that in MG group, especially in MA and HA groups, Specific information was given in Supplementary Figure 1 and Supplementary Table 1. Histopathological results showed that the gastric glands were closely arranged and regular, and the epithelial cells remained intact without congestion or edema in the NG group.

Table 1 Sequences of the primers for real-time reverse transcription polymerase chain reaction

Gene	Forward primer	Reverse primer	Length
EGFR	CCTATGGGCCAAAGATCCCA	GAGGTTCCACGAGCTCTCTC	162 bp
ZO-1	CACCACAGACATCCAACCAG	CACCAACCACTCTCCCTTGT	230 bp
NF-κBp65	AGGCCATTGAAGTGATCCAG	CAGTGAGGGACTCCGAGAAG	204 bp
IκBα	CACGGAAGATGAGTTGCCCT	CAAGTCCACGTTCCTTTGGC	91 bp
GAPDH	AGACAGCCGCATCTTCTTGT	CTTGCCGTGGGTAGAGTCAT	207 bp

EGFR: Epidermal growth factor receptor; ZO-1: Zonula Occludens-1; NF-κB: Nuclear factor kappa-B; IκBα: IkappaBalpha.

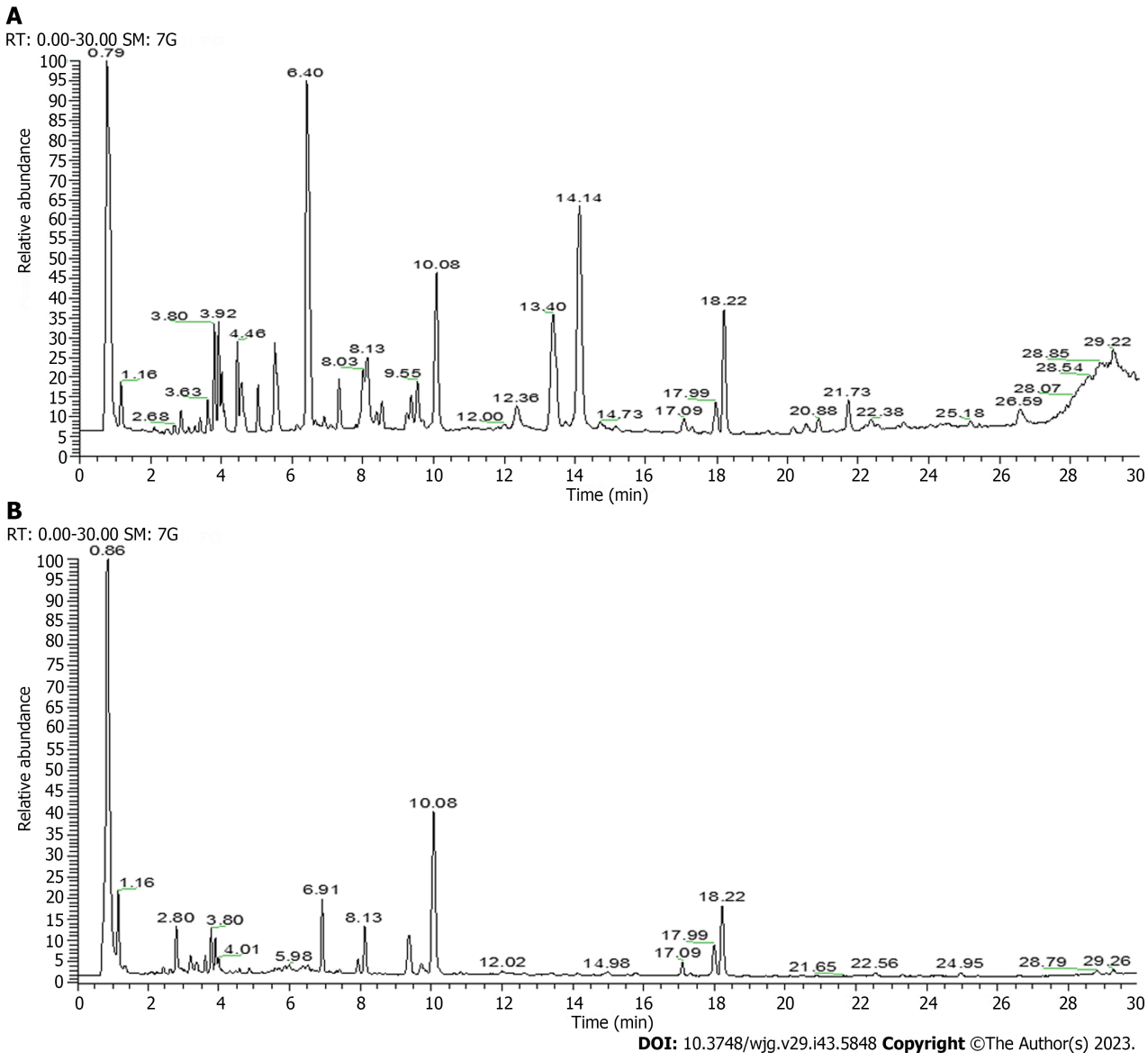

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Figure 1 Total ion current chromatogram of *Atractylodes japonica* by liquid chromatography-tandem mass spectrometry. A: Positive ion mode; B: Negative ion mode.

Compared with the NG group, the epithelial cells of the gastric mucosa were disorganized with edema and inflammatory cell infiltration in the MG group, showing pathological changes. The histopathology associated with GU was improved, and inflammatory cell infiltration was reduced by the intervention of positive control drug and *A. japonica*, especially in the HA group (Figure 2).

Table 2 Identification of chemical constituents of *Atractylodes japonica* by UPLC Q-Exactive Orbitrap MS

No.	tR/min	Compound	Molecular formula	Adduct ion	Theoretical (m/z)	Measured (m/z)	Error (ppm)
1	0.83	Asparagine	$C_4H_8N_2O_3$	$[M+H]^+$	133.06076	133.06033	-3.297
2	0.85	Sucrose	$C_{12}H_{22}O_{11}$	[M−H+HCOOH]	387.11441	387.11374	-1.741
3	0.86	Manninotriose	$C_{18}H_{32}O_{16}$	$[M-H]^-$	503.16092	503.16175	-1.665
4	0.85	Valine	$C_5H_{11}NO_2$	$[M+H]^+$	118.08625	118.08591	-2.923
5	0.87	5-hydroxymethylfurfural	$C_6H_6O_3$	$[M+H]^+$	127.03897	127.03864	-2.602
6	0.91	Niacinamide	$C_6H_6N_2O$	$[M+H]^+$	123.05529	123.05509	-1.620
7	1.19	Pyroglutamic acid	$C_5H_7NO_3$	$[M+H]^+$	130.04986	130.04953	-2.612
8	1.25	Tyrosine	$C_9H_{11}NO_3$	$[M+H]^+$	182.08116	182.08063	-2.964
9	1.27	Citric acid	$C_6H_8O_7$	$[M-H]^-$	191.01972	191.01880	-4.847
10	1.35	Isoleucine	$C_6H_{13}NO_2$	$[M+H]^+$	132.10190	132.10153	-2.840
11	1.46	Leucine	$C_6H_{13}NO_2$	$[M+H]^+$	132.10190	132.10153	-2.840
12	2.10	Phenylalanine	$C_9H_{11}NO_2$	$[M+H]^+$	166.08625	166.08577	-2.921
13	2.25	Neochlorogenic acid	$C_{16}H_{18}O_9$	$[M-H]^-$	353.08781	353.08783	0.050
14	2.44	Chlorogenic acid	$C_{16}H_{18}O_9$	$[M-H]^-$	353.08781	353.08752	-0.808
15	2.44	Atractyloside A	$C_{21}H_{36}O_{10}$	[M−H+HCOOH]	493.22905	493.22864	-0.831
16	2.80	Cryptochlorogenic acid	$C_{16}H_{18}O_9$	$[M-H]^-$	353.08781	353.08728	-1.488
17	2.86	Caffeic acid	$C_9H_8O_4$	$[M+H]^+$	181.04953	181.04890	-3.509
18	2.86	7-hydroxycoumarin	$C_9H_6O_3$	$[M+H]^+$	163.03897	163.03841	-3.439
19	3.17	Catechin	$C_{15}H_{14}O_6$	$[M-H]^-$	289.07176	289.07172	-0.143
20	3.19	Isochlorogenic acid B	$C_{25}H_{24}O_{12}$	$[M-H]^-$	515.11950	515.11890	-1.163
21	3.27	Isoorientin	$C_{21}H_{20}O_{11}$	$[M+H]^+$	449.10784	449.10669	-2.556
22	3.51	Rutin	$C_{27}H_{30}O_{16}$	$[M-H]^-$	609.14610	609.14630	0.315
23	3.52	Hyperoside	$C_{21}H_{20}O_{12}$	$[M-H]^-$	463.08819	463.08826	0.131
24	3.52	Quercetin	$C_{15}H_{10}O_7$	$[M+H]^+$	303.04992	303.04895	-3.528
25	3.68	Kaempferol-3-O- rutinoside	$C_{27}H_{30}O_{15}$	$[M-H]^-$	593.15119	593.15125	0.096
26	3.77	Isorhamnetin 3-O-neohesperidin	$C_{28}H_{32}O_{16}$	$[M-H]^-$	623.16176	623.16107	-1.104
27	3.78	Narcissoside	$C_{28}H_{32}O_{16}$	$[M+H]^+$	625.17631	625.17371	-4.161
28	3.78	Isorhamnetin	$C_{16}H_{12}O_7$	$[M+H]^+$	317.06558	317.06421	-4.318
29	3.83	Vanillin	$C_8H_8O_3$	$[M+H]^+$	153.05462	153.05406	-3.663
30	3.86	Isochlorogenic acid A	$C_{25}H_{24}O_{12}$	$[M-H]^-$	515.11950	515.11914	-0.697
31	3.96	Isochlorogenic acid C	$C_{25}H_{24}O_{12}$	$[M-H]^-$	515.11950	515.11896	-1.047
32	6.40	Palmitic acid	$C_{16}H_{32}O_2$	$[M+NH_4]^+$	274.27406	274.27292	-4.141
33	6.47	Cinnamyl alcohol	$C_9H_{10}O$	$[M+H]^+$	135.08044	135.07999	-3.343
34	6.51	Curcumenol	$C_{15}H_{22}O_2$	$[M+H]^+$	235.16925	235.16826	-4.237
35	7.34	Atractylenolide III	$C_{15}H_{20}O_3$	$[M+H]^+$	249.14852	249.14752	-4.018
36	7.62	Atractylenolactam	$C_{15}H_{19}NO$	$[M+H]^+$	230.15394	230.15340	-2.350
37	9.24	3β-hydroxyatractylone	$C_{15}H_{20}O_2$	$[M+H]^+$	233.15361	233.15279	-3.502
38	9.55	Atractylenolide II	$C_{15}H_{20}O_2$	$[M+H]^+$	233.15361	233.15274	-3.716
39	12.00	Atractylenolide VI	$C_{15}H_{22}$	$[M+H]^+$	203.17943	203.17889	-2.644

40	12.01	α-linolenic acid	$C_{18}H_{30}O_2$	$[M+H]^+$	279.23185	279.23108	-2.782
41	12.14	3β-acetoxyatracty	$C_{17}H_{22}O_3$	$[M+H]^+$	275.16417	275.16333	-3.057
42	12.38	Atractylenolide I	$C_{15}H_{18}O_2$	$[M+H]^+$	231.13796	231.13721	-3.229
43	12.95	Atractylenolide V	$C_{15}H_{20}O_4$	$[M+H]^+$	265.14343	265.14258	-3.227
44	13.42	(6E,12E)-tetradecadiene-8,10-diyne-1,3-diol diacetate	$C_{18}H_{22}O_4$	$[M+H]^+$	303.15908	303.15805	-3.416
45	13.97	β-elemene	$C_{15}H_{24}$	$[M+H]^+$	205.19508	205.19443	-3.155
46	14.14	Eudesma-4(15),7(11)-dien-8-one	$C_{15}H_{22}O$	$[M+H]^+$	219.17434	219.17346	-4.024
47	20.55	Atractylon	$C_{15}H_{20}O$	$[M+H]^+$	217.15869	217.15800	-3.186
48	21.83	Biatractylolide	$C_{30}H_{38}O_4$	$[M+H]^+$	463.28428	463.28290	-2.992

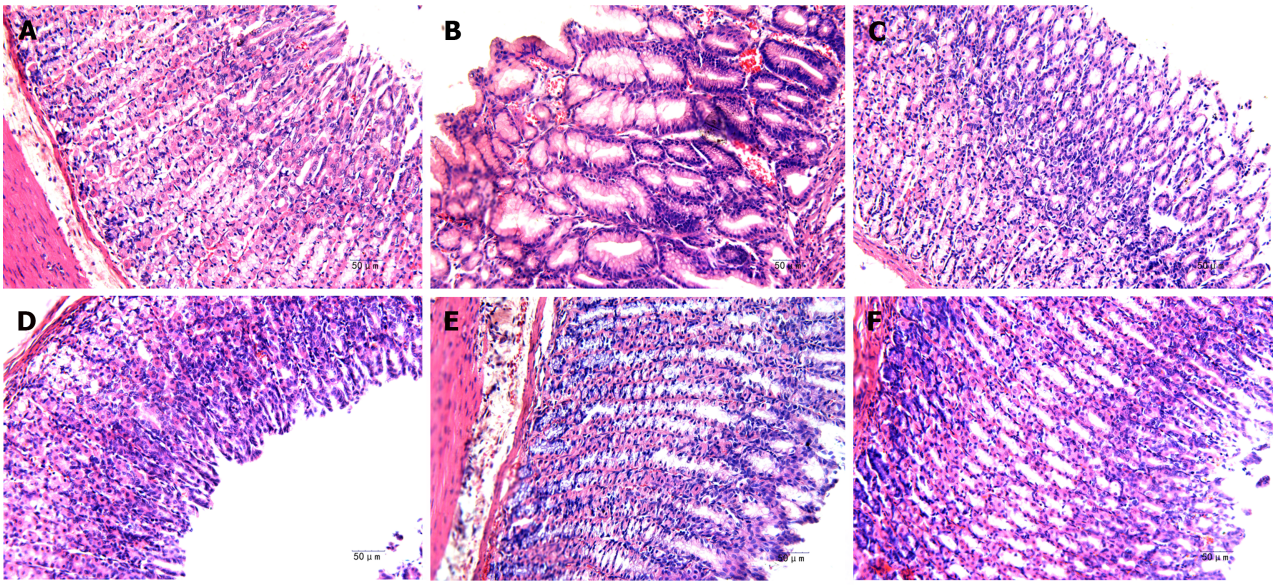

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Figure 2 Effect of *Atractylodes japonica* on pathological changes of gastric tissues in gastric ulcer rats after 10 d of treatment (*n* = 8). A-F: Histological analysis was performed by hematoxylin and eosin staining (200 × original magnification, scale bar 50 μm). Normal group (A); acetic acid-induced model group (B); omeprazole group (C); low dose group of *Atractylodes japonica* (*A. japonical*) (D); middle dose group of *A. japonica* (E); high dose group of *A. japonica* (F).

Effects of A. japonica on the levels of EGF, EGFR, NF-κB, IL-1β, IL-10 and NKA in serum and gastric tissues

The levels of EGF, EGFR, NF-κB, IL-1β, IL-10, and NKA in the serum and gastric tissues of rats were detected by ELISA. The results showed that the levels of EGF, EGFR, IL-10, and NKA in the MG group were significantly lower ($P < 0.01$), while the levels of NF-κB and IL-1β were significantly higher than in the NG group ($P < 0.01$). Compared with the MG group, the HA group could significantly increase EGF, EGFR, IL-10, and NKA levels and decrease the levels of NF-κB and IL-1β in the serum and gastric tissues ($P < 0.05$ or $P < 0.01$). In addition, compared with the MG group, the levels of EGF and EGFR increased and IL-1β decreased in the MA group's serum ($P < 0.05$ or $P < 0.01$). The levels of EGFR increased in the MA and LA groups ($P < 0.01$) and IL-1β decreased in the MA group of gastric tissues ($P < 0.05$) (Figures 3 and 4).

Effects of A. japonica on the mRNA expressions of EGFR, Zonula Occludens-1, NF-κBp65 and IkappaBalpha in gastric tissues

The mRNA expressions of EGFR, Zonula Occludens-1 (ZO-1), and IkappaBalpha (IκBα) in the MG group were significantly lower ($P < 0.01$), while the expression of NF-κBp65 was significantly higher than in the NG group ($P < 0.01$). Compared with the MG group, the mRNA expressions of EGFR, ZO-1, and IκBα significantly decreased, while the expression of NF-κBp65 significantly increased in the HA group ($P < 0.05$). In addition, the expressions of EGFR and IκBα could also increase in MA and LA groups ($P < 0.01$) (Figure 5).

MVA

The multivariate pattern recognition analysis was first analyzed by PCA, an unsupervised learning method. The results of the PCA score plot including the QC samples showed a certain trend of separation among the different groups (Figure 6), where tightly aggregated QC samples indicated MS platform was stable. Next, the plasma metabolism

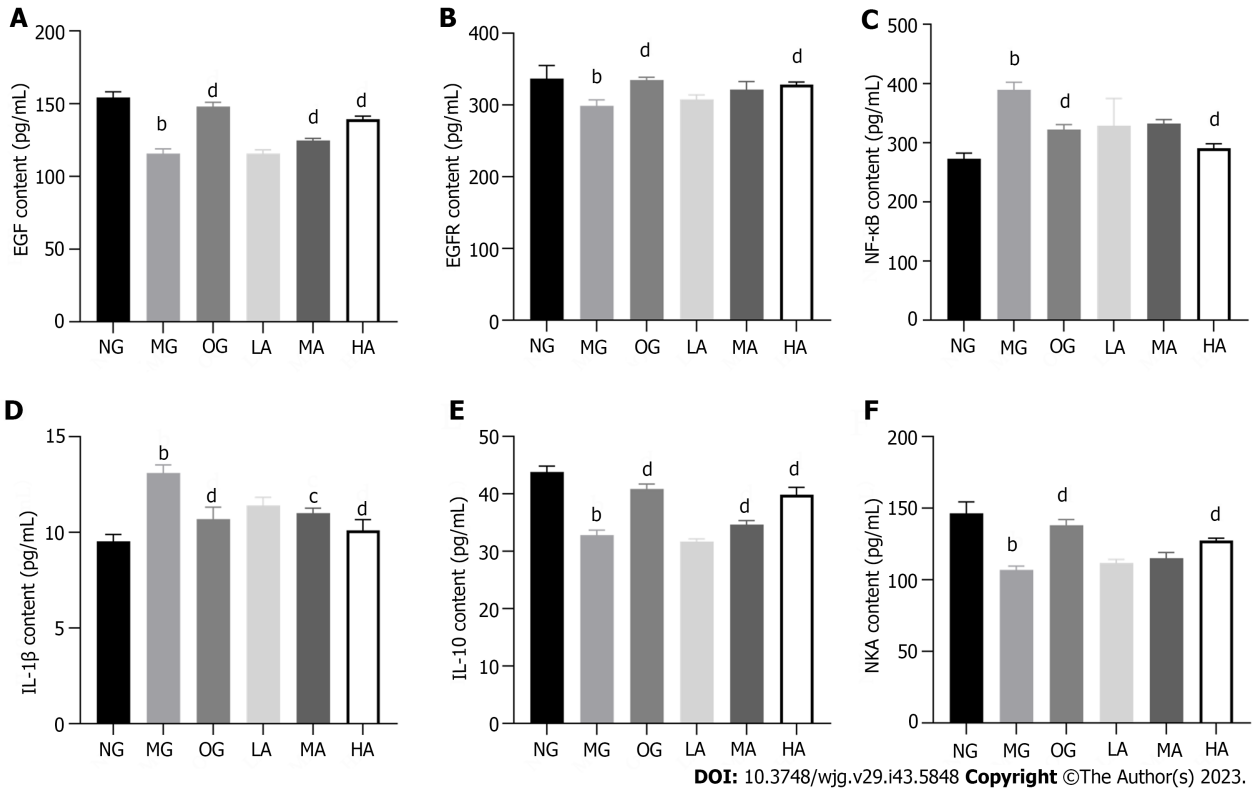

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Figure 3 Effect of *Atractylodes japonica* on gastric ulcer-associated factors level in serum of gastric ulcer rats (*n* = 8). A-F: The epidermal growth factor (EGF), EGF receptor, nuclear factor kappa-B, interleukin-1β (IL-1β), IL-10, and Na^+-K^+-ATPase levels were detected by enzyme-linked immunosorbent assay in serum of gastric ulcer rats. $^bP < 0.01$ *vs* normal group; $^cP < 0.05$ and $^dP < 0.01$ *vs* model group. EGF: Epidermal growth factor; EGFR: Epidermal growth factor receptor; NF-κB: Nuclear factor kappa-B; IL: Interleukin; NKA: Na^+-K^+-ATPase; NG: Normal group; MG: Model group; OG: Omeprazole group; LA: Low dose group of *Atractylodes japonica*; MA: Middle dose group of *Atractylodes japonica*; HA: High dose group of *Atractylodes japonica*.

between NG and MG groups and between MG and HA groups were analyzed by OPLS-DA, a supervised discriminant analysis statistical method. It was observed that there was a clear trend of separation between different groups (Figures 7A and C). The permutation test results (values of R2 and Q2) also showed that the model was stable and reliable (Figures 7B and D).

Biomarker screenings and pathway enrichment analysis

A total of 10 endogenous metabolites were identified as potential biomarkers by database analysis (Table 3). The heat map directly reflected the differences in the expression of the relative abundance of metabolites among groups (Figure 8A). MetaboAnalyst 5.0 was used for enrichment analysis of potential biomarkers, and the main metabolic pathways associated with pathway enrichment analysis included arginine biosynthesis, primary bile acid biosynthesis, taurine and hypotaurine metabolism, glycerophospholipid metabolism, arginine and proline metabolism, purine metabolism, steroid hormone biosynthesis (Table 4 and Figure 8B).

DISCUSSION

The acetic acid-induced ulcer model is one of the most commonly used models. Since its development in 1969, it has been widely recognized in the scientific community. Its pathological morphology and repair process are similar to human GUs, with the advantages of easy induction process, good model repeatability, and a high survival rate[19,26]. Consequently, the findings of this study demonstrated that *A. japonica* had a therapeutic impact on gastrointestinal ulcers, with improved recovery outcomes observed across multiple related indexes. According to pathological sections, the ulcer status of rats was significantly improved.

Since metabolites are downstream products of gene and protein expression, they can respond more quickly and provide effective information when the organism is affected[27]. MVA is characterized by the analysis of the statistical rules of multiple interrelated research objects, monitoring variables, and focusing on the internal changes of variables. Therefore, it is widely used in metabolomics research, such as biomarker selection through the construction of models. The research fields cover animals, plants, medicine, the environment, *etc*[28-31]. Typically, the MVA is divided into supervisory and non-supervisory methods. At present, the most commonly used unsupervised recognition mode is PCA [32], which maximizes the extraction of the original information while reducing the dimension of the data. If PCA is not successful in distinguishing subtle differences between sample groups, the supervised models PLS-DA and OPLS-DA can

Table 3 Identified metabolites of plasma

No.	Compound name	R.T. (min)	Formula	Exact mass (m/z)	HMDB ID	MG *vs* NG	*P* value	HA *vs* MG	*P* value
1	L-arginine	0.77	$C_6H_{14}N_4O_2$	174.1116	HMDB0000517	Decreased	< 0.01	Increased	< 0.05
2	Citrulline	0.79	$C_6H_{13}N_3O_3$	175.0956	HMDB0000904	Decreased	< 0.01	Increased	< 0.05
3	Taurine	0.79	$C_2H_7NO_3S$	125.0146	HMDB0000251	Decreased	< 0.05	Increased	< 0.05
4	Adenine	1.42	$C_5H_5N_5$	135.0545	HMDB0000034	Increased	< 0.05	Decreased	< 0.05
5	Glycocholic acid	6.31	$C_{26}H_{43}NO_6$	465.3090	HMDB0000138	Decreased	< 0.05	Increased	< 0.05
6	Aldosterone	7.59	$C_{21}H_{28}O_5$	360.1936	HMDB0000037	Decreased	< 0.05	Increased	< 0.05
7	Glycochenodeoxycholic acid	8.09	$C_{26}H_{43}NO_5$	449.3138	HMDB0000637	Decreased	< 0.05	Increased	< 0.05
8	LysoPC (16:0/0:0)	10.17	$C_{24}H_{50}NO_7P$	495.3324	HMDB0010382	Increased	< 0.01	Decreased	< 0.01
9	LysoPC (18:0/0:0)	11.20	$C_{26}H_{54}NO_7P$	523.3637	HMDB0010384	Increased	< 0.05	Decreased	< 0.05
10	Eicosadienoic acid	13.76	$C_{20}H_{36}O_2$	308.2715	HMDB0005060	Increased	< 0.01	Decreased	< 0.05

MG: Model group; NG: Normal group; HA: High dose group of the crude *Atractylodes japonica*.

Table 4 Results of enrichment analysis of biomarkers

No.	Pathway name	Match status	*P* value	-Log (*P*)	Impact
1	Primary bile acid biosynthesis	3/46	0.0012354	2.9082	0.0254
2	Arginine biosynthesis	2/14	0.0020576	2.6866	0.30457
3	Taurine and hypotaurine metabolism	1/8	0.040642	1.391	0.42857
4	Glycerophospholipid metabolism	1/36	0.17174	0.76513	0.01736
5	Arginine and proline metabolism	1/38	0.18047	0.74359	0.05786
6	Purine metabolism	1/65	0.29073	0.53651	0.00528
7	Steroid hormone biosynthesis	1/85	0.3638	0.43913	0.01032

be used to maximize the degree of separation between sample groups. The drawback is that data can be overfitted. Therefore, strict cross-validation must be carried out to ensure the reliability of the model. The differentiated metabolites obtained by screening often have functional similarities or complementarity in biology or participate in positive or negative regulation of the same metabolic pathway, which is manifested as similar or opposite expression characteristics between different experimental groups. Therefore, cluster analysis of these characteristics is helpful to speculate on the function of metabolites and explore the mechanism of disease treatment. This study made full use of MVA for metabonomic data analysis in order to make the experimental results more accurate and reliable[33].

Liquid chromatography-mass spectrometry technology has been widely used for the identification and analysis of traditional Chinese medicine or complex unknown substances. In this study, technology was used to analyze the extract of *A. japonica*. Among the identified components, multiple of them have potential biological activity. Terpenoids are the most common active components, of which atractylon and atractylenolide I, II, and III have anti-inflammatory effects and regulate gastrointestinal function[34,35]. The organic acid component chlorogenic acid[36] has obvious antioxidant, antibacterial, anti-inflammatory, antiviral, and other effects. Isoorientin[37], a flavonoid component, has anti-oxidative and anti-inflammatory effects, regulates the intestinal flora, *etc*. These components may be closely related to the treatment of GU and the potential key pharmacological bases of *A. japonica*.

GU is the most common cause of gastric mucosal inflammation and injury. NF-κB is a primary regulator of the inflammatory response, and one of its subunits, NF-κBp65 is the main functional protein. IκB, as a suppressor protein of the NF-κB signaling pathway, when stimulated by injury factors such as ulcers, will activate NF-κB, leading to the degradation of IκB, and subsequently promoting the expression of multiple inflammatory factors[38-40]. Cytokines IL-1β and IL-10 are common indicators of acute inflammation and are closely related to the severity of GUs[41,42]. IL-1β, as a pro-inflammatory factor, regulates the function of various gastric epithelial cells and interacts with NF-κB to cause the release of inflammatory mediators and stimulate the secretion of other cytokines, exacerbating the inflammatory response[43]. IL-10 can inhibit the pro-inflammatory response and limit unnecessary tissue destruction caused by inflammation[44]. The results showed that the level of IL-1β and expression of NF-κBp65 were significantly increased, while the level of IL-10 and expression of IκBα were significantly decreased in GU rats, suggesting that the progression of GU is closely related to inflammation. After the intervention of *A. japonica*, the expression levels of inflammatory factors were significantly re-regulated, which is in accordance with the research results of Hu *et al*[45] and Zhou *et al*[46]. Simultaneously, pathological

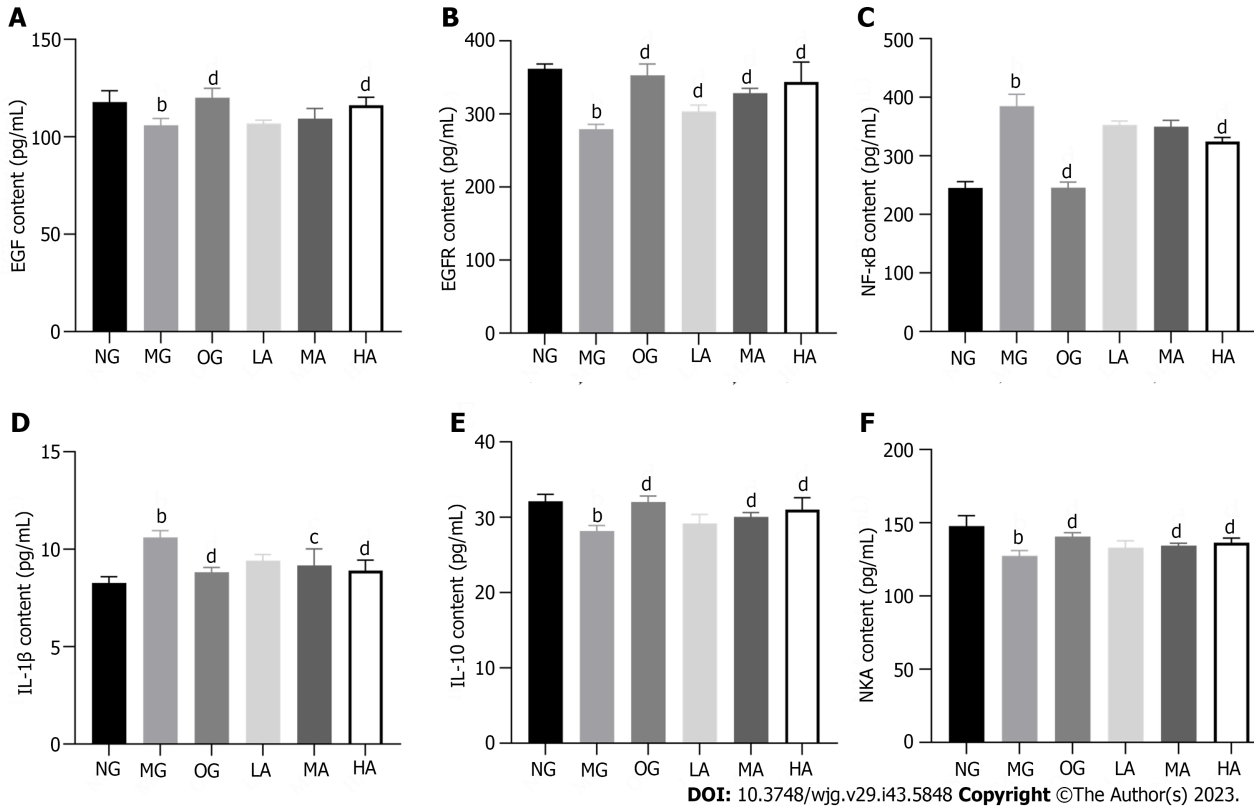

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Figure 4 Effect of *Atractylodes japonica* on gastric ulcer-associated factors level in gastric tissues of gastric ulcer rats (*n* = 8). A-F: The epidermal growth factor (EGF), EGF receptor, nuclear factor kappa-B, interleukin-1β (IL-1β), IL-10, and Na^+-K^+-ATPase levels were detected by enzyme-linked immunosorbent assay in gastric tissues of gastric ulcer rats. $^bP < 0.01$ *vs* normal group; $^cP < 0.05$ and $^dP < 0.01$ *vs* model group. EGF: Epidermal growth factor; EGFR: Epidermal growth factor receptor; NF-κB: Nuclear factor kappa-B; IL: Interleukin; NKA: Na^+-K^+-ATPase; NG: Normal group; MG: Model group; OG: Omeprazole group; LA: Low dose group of *Atractylodes japonica*; MA: Middle dose group of *Atractylodes japonica*; HA: High dose group of *Atractylodes japonica*.

results showed *A. japonica* had a significant protective effect on GU tissue and reduced inflammatory damage to the gastric mucosa. All these results suggest that the gastroprotective effect of *A. japonica* is related to the inhibition of the inflammatory response.

On the other hand, GU causes mucosal damage and destroys the structure and function of the gastric mucosa. EGF is an endogenous substance that can inhibit gastric acid secretion, promote epithelial cell proliferation, tissue repair, and cytoprotection, and is an important factor in promoting wound healing. Its receptor EGFR also plays an important role in cell proliferation and other effects, both of which generally combine and activate downstream effectors to promote the repair and healing of injured mucosa[47,48]. ZO-1 is a bridging protein, which is an important component of tight junctions; most of them are located at the junctions between cells. It mainly interacts with extracellular signal transduction pathways and the cytoskeleton, and the normal expression of ZO-1 is closely related to mucosal integrity[49, 50]. It plays an important role not only in regulating the transport of intracellular substances and maintaining epithelial polarity but also in cell proliferation and differentiation. According to the results, the ulcer may destroy the integrity and permeability of the gastric mucosa, leading to the degradation of the protein structure[51]. NKA is a ubiquitous transmembrane protein that maintains the normal function of mucosal cells and membrane permeability and maintains Na^+ and K^+ gradients across the cell membrane through energy from adenosine triphosphate (ATP). External stimulation will cause abnormal function of this enzyme and mucosal damage[52]. According to the results, the expression levels of EGF, EGFR, and ZO-1 were significantly decreased in GU rats, suggesting that GU leads to impaired mucosal barrier function and disrupted structure. After intervention, the expression levels of all factors were significantly increased, indicating that *A. japonica* could promote ulcer healing and improve gastric mucosal function. Based on the above pharmacodynamic results, it was found that the HA group had the best effect among the treatment groups, so the HA group was selected for further metabolic analysis.

The metabolomic study showed that GUs are involved in multiple metabolic pathways. Amino acid metabolism: L-arginine has a variety of biological effects, including gastric protection and promoting ulcer healing properties. Its metabolite, citrulline, has been confirmed to protect gastric mucosa from ulcer-induced mucosal damage and regulate mucosal integrity[53,54]. Taurine is also a cytoprotective factor, maintaining the storage of an important antioxidant and free radical scavenger glutathione in the body, increasing membrane stability, and preventing inflammation and gastric mucosal damage[55,56]. According to the results, the levels of amino acids decreased in the MG group, which indicates an amino acid metabolic disorder; however, *A. japonica* can significantly recover the levels of amino acids.

Lipid metabolism: Multiple experimental studies have shown that lysophosphatidylcholines are a class of metabolites associated with inflammatory damage caused by GU, which can induce impairment of gastric mucosal barrier function,

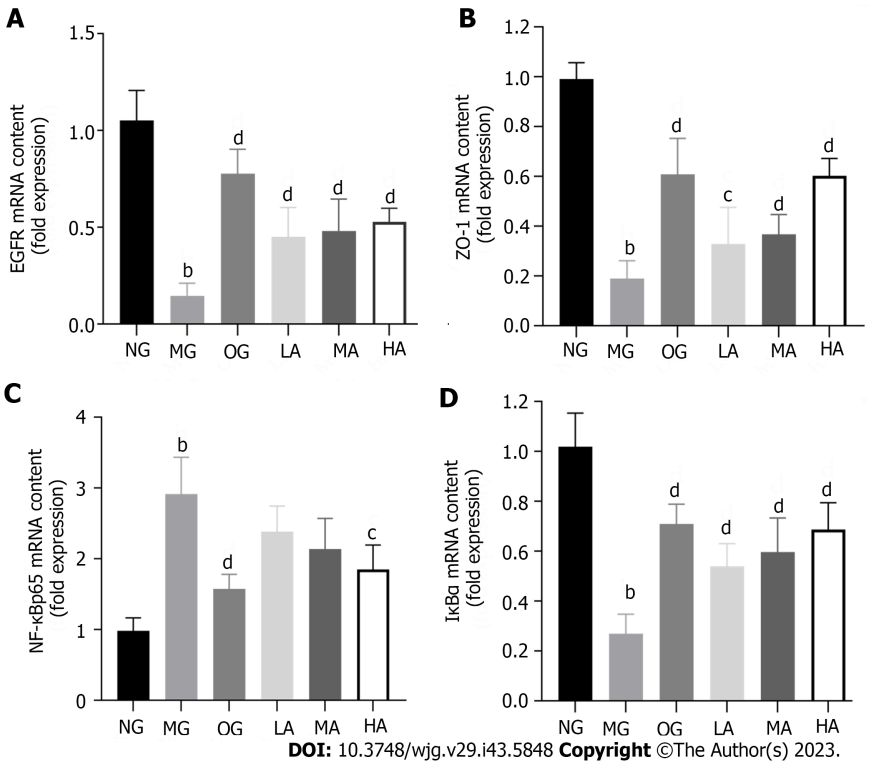

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Figure 5 Effect of *Atractylodes japonica* on gastric ulcer-associated genes in acid-induced gastric tissues (*n* = 8). A-D: The mRNA levels of epidermal growth factor receptor, Zonula Occludens-1, nuclear factor kappa-B p65, IkappaBalpha in gastric tissues were analysed by real-time reverse transcription polymerase chain reaction. $^{b}P < 0.01$ *vs* normal group; $^{c}P < 0.05$ and $^{d}P < 0.01$ *vs* model group. EGFR: Epidermal growth factor receptor; ZO-1: Zonula Occludens-1; NF-κB: Nuclear factor kappa-B; IκBα: IkappaBalpha; NG: Normal group; MG: Model group; OG: Omeprazole group; LA: Low dose group of *Atractylodes japonica*; MA: Middle dose group of *Atractylodes japonica*; HA: High dose group of *Atractylodes japonica*.

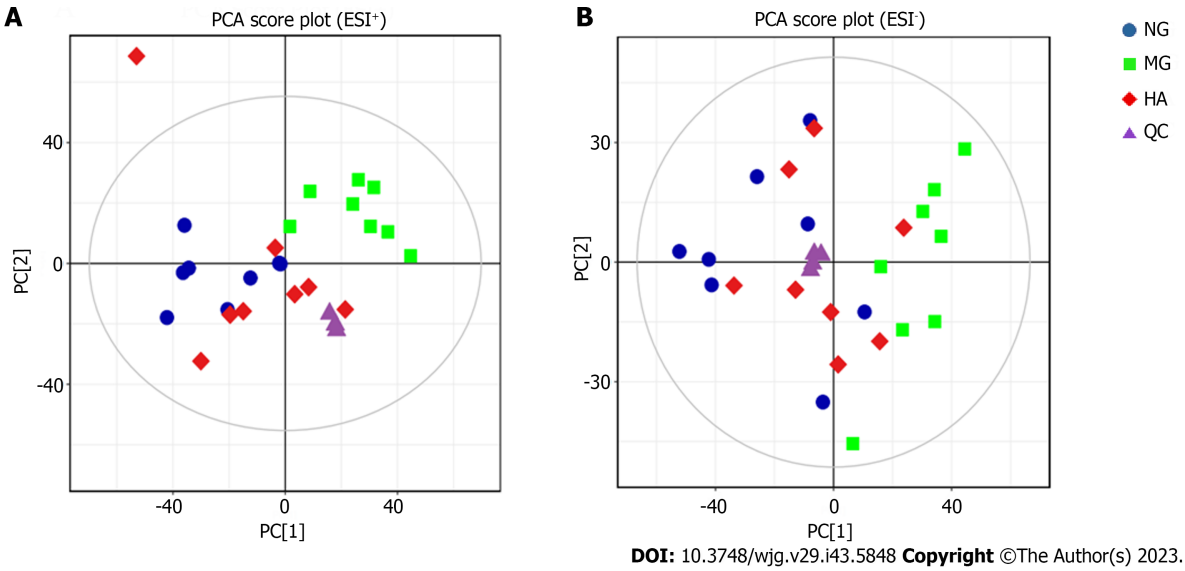

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Figure 6 Principal component analysis score plots of plasma metabolomics analysis (*n* = 8). Principal component analysis score plots among normal group, model group, high dose group of *Atractylodes japonica* and quality control groups. A: Positive ion mode(ESI^{+}); B: Negative ion mode(ESI^{-}). PCA: Principal component analysis; NG: Normal group; MG: Model group; HA: High dose group of *Atractylodes japonica*; QC: Quality control.

leading to gastric mucosal damage[57,58]. Eicosadienoic acid is an n-6 polyunsaturated fatty acid with certain pro-inflammatory activity, which can not only metabolize to the eicosanoid precursor compound arachidonic acid to promote an inflammatory response but also promote the expression of inflammatory mediators such as NF-κBp65 to cause mucosal inflammatory damage[59,60]. Another study has found that eicosadienoic acid can be positively associated with ulcerative colitis[61]. Furthermore, since reactive oxygen species (ROS) is an important factor in the early stage of inflammation and mediates the onset of inflammation, eicosadienoic acid can act by reducing ROS level induced by lipopolysaccharide and was presumed to have potential anti-inflammatory activity to some extent[62]. The dualism effect of this

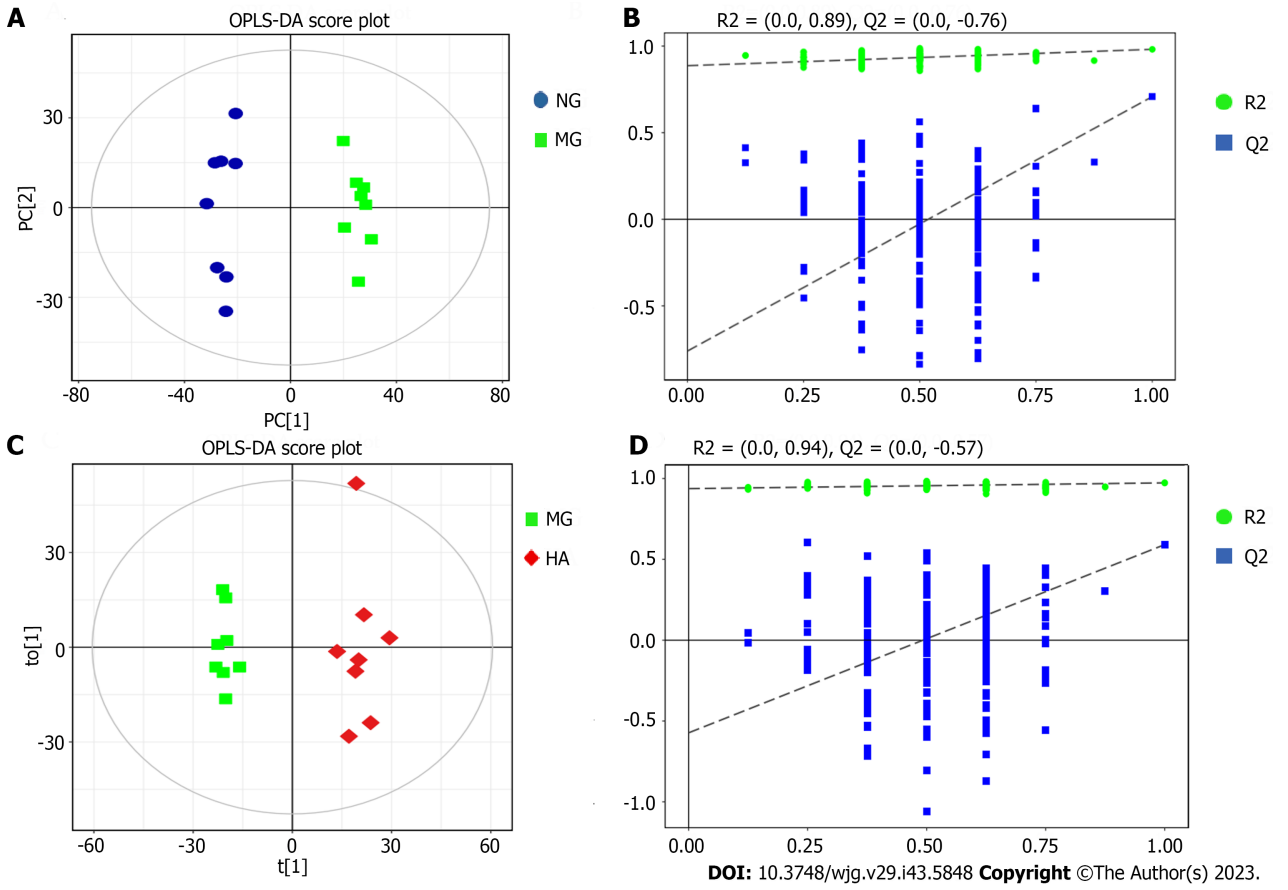

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Figure 7 Orthogonal projections to latent structures-discriminant analysis score plots and 200-permutation test of plasma metabolomics analysis (*n* = 8). A: Orthogonal projections to latent structures-discriminant analysis (OPLS-DA) score plots between normal group (NG) and model group (MG) groups; B: 200-permutation test between NG and MG groups; C: OPLS-DA score plots between MG and high dose group of *Atractylodes japonica* (HA) groups; D: 200-permutation test between MG and HA groups. OPLS-DA: Orthogonal projections to latent structures-discriminant analysis; MG: Model group; HA: High dose group of *Atractylodes japonica*.

metabolite may be related to the disease and the course of disease. It is worth further exploring. According to the results, the levels were significantly increased in the MG group, suggesting that lipid metabolism disorder is one of the metabolic pathways exacerbating ulcers, and the levels recovered after intervention.

Bile acids are a class of cholesterol derivatives that regulate a variety of metabolic and inflammatory pathways[63]. We found that the levels of glycochenodeoxycholic acid and glycocholic acid in the MG decreased significantly in this study. According to existing reports, glycochenodeoxycholic acid promotes cell proliferation in a concentration-dependent manner through activation of Gαi, reduction of cyclic AMP, and an increase in H2AX phosphorylation[64]. The Farnesoid X receptor (FXR), also known as the bile acid receptor, regulates lipid metabolism, mitigates the inflammatory response, and enhances barrier function. Some studies showed that glycocholic acid can improve tissue growth performance, reduce tissue damage, and play a protective role in mucous membranes by activating FXR[65]. In our study, the levels of two kinds of cholic acid were significantly reduced in GU rats. It is hypothesized that ulcers may disrupt barrier function and activate the inflammatory response by interfering with the bile acid metabolic pathway. Treatment with *A. japonica* significantly alleviates the inflammatory response and improves metabolite levels. Stepien *et al*[66] have shown that these two cholic acids increased in a variety of liver diseases, such as hepatocellular carcinoma. Aragonès *et al*[67] found that the level of glycocholic acid was significantly elevated in patients with non-alcoholic fatty liver disease. It is suggested that the same metabolite may have opposite tendency for different diseases.

The adrenal cortex produces the steroid hormone aldosterone, which regulates electrolyte and water balance by increasing sodium renal retention and potassium excretion. There are a few studies on aldosterone-related GU. Matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) are closely related to ulcers. MMPs belong to a class of endogenous proteolytic enzymes that generally aggravate the inflammatory response and prolong healing time. TIMPs are endogenous specific suppressors of MMPs, which play a role in the process of ulcer healing by inhibiting and regulating MMPs[68]. Studies have shown that aldosterone can induce TIMP-1 secretion and promote collagen accumulation[69]. It is speculated that ulcers interfered with aldosterone production and that downstream factors could not play a healing role. However, treatment with *A. japonica* alleviated inflammation and promoted ulcer healing.

Adenine is a component of adenosine. When adenosine reacts with three phosphate groups, it forms the nucleotide ATP, the body's most direct source of energy. Adenine increased in the MG group, suggesting that ulcers damaged the body's energy metabolism, resulting in blocked ATP synthesis and adenine accumulation. When *A. japonica* intervened to restore the process of energy metabolism, the levels of adenine decreased.

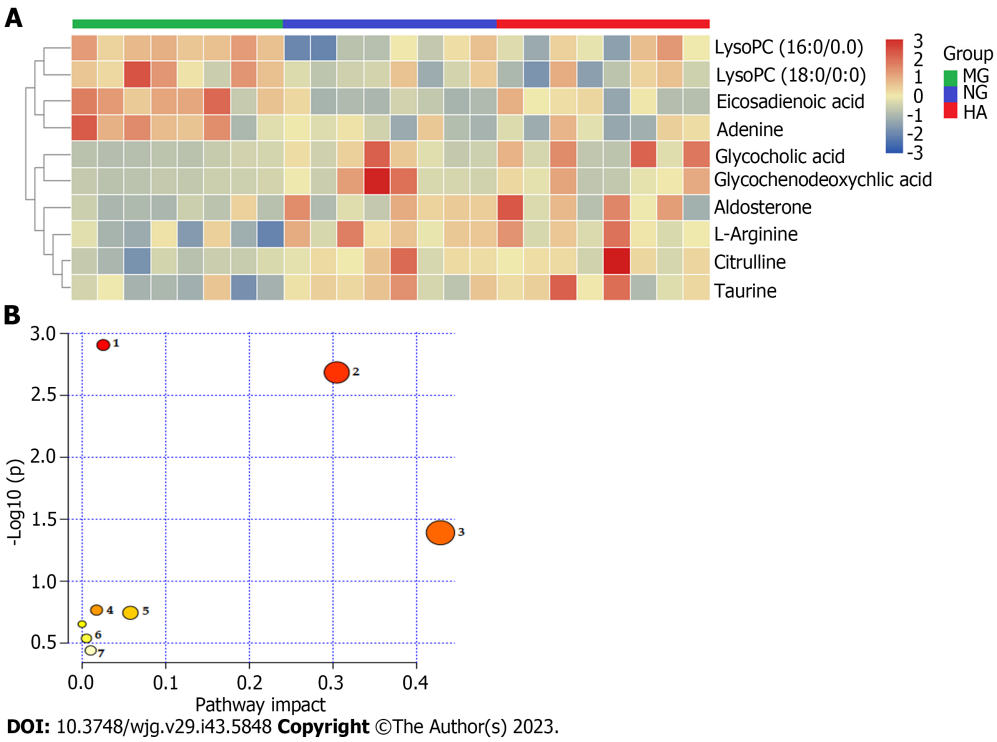

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Figure 8 Enrichment analysis of metabolic markers (*n* = 8). A: Heatmap of potential biomarkers and the degree of the changes are marked in red (up-regulation) and blue (down-regulation); B: Metabolic pathways involved in the therapeutic effects of *Atractylodes japonica* on gastric ulcer. NG: Normal group; MG: Model group; HA: High dose group of *Atractylodes japonica*.

CONCLUSION

In this study, 48 potential bioactive compounds were identified by UPLC–MS/MS that may be active components of GU. Additionally, the pathogenesis of acetic acid-induced GU in rats and the therapeutic effect of *A. japonica* were explored from the perspective of metabonomics for the first time. The results showed that *A. japonica* could correct the metabolic disorder of GU with its gastroprotective effect and effectively relieve mucosal inflammatory injury. The specific anti-ulcer effect is closely related to the anti-inflammatory activity produced by down-regulating NF-κB and IL-1β and up-regulating IL-10 and IκBα, as well as the gastric protective effect produced by up-regulating EGF, EGFR, ZO-1, and NKA. Combined with omics enrichment of metabolic pathways, we found that lipid metabolism, amino acid metabolism, and other metabolic pathways play a protective role in the stomach through anti-inflammatory, antioxidative, ulcer healing, and other functions. This study provides a research direction for the potential mechanism of *A. japonica* in the treatment of GU, which is of great significance for its drug development and clinical application.

ARTICLE HIGHLIGHTS

Research background

Gastric ulcer (GU) is a common digestive system disease. In addition to western medicine treatment, more and more Chinese herbs have become the first choice for alternative treatment due to their long history of use, high efficacy, low side effects and low price.

Research motivation

Although the herbal medicine *Atractylodes japonica* Koidz. ex Kitam. (*A. japonica*) has an obvious therapeutic effect on GU, there are few relevant mechanism studies at present.

Research objectives

The object of this study is to investigate the protective effects of *A. japonica* on acetic acid-induced GU rats.

Research methods

We used ultra performance liquid chromatography tandem mass spectrometry, hematoxylin-eosin stain, enzyme-linked immunosorbent assay, real-time reverse transcription polymerase chain reaction and acetic acid-induced GU model to evaluate the therapeutic effect of *A. japonica*.

Research results

48 chemical constituents of *A. japonica* were identified, the herb significantly improved the pathological damage of gastric tissues, increased the expression levels of anti-inflammatory factors, decreased the expression levels of proinflammatory factors, restored the levels of factors about ulcer healing and energy metabolism. and identified 10 potential differential metabolites and enriched 7 related metabolic pathways of metabolomic analysis.

Research conclusions

The therapeutic effect of *A. japonica* on GU rats is closely related to anti-inflammation and repair of gastric injury, and is regulated and treated through a combination of multiple pathways.

Research perspectives

These findings contribute to the understanding of the potential mechanism of *A. japonica* to improve acetic acid-induced GU, and will provide great importance for the development and clinical application of *A. japonica.*

FOOTNOTES

Author contributions: Li F and Cai Q designed the experiment; Zhen BX completed the whole experiment and wrote the manuscript; and all authors approved the final version of the article.

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Country/Territory of origin: China

ORCID number: Bi-Xian Zhen 0000-0001-7497-2759; Qian Cai 0000-0001-6746-5513; Feng Li 0000-0002-8455-622X.

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CASE REPORT

Pediatric-type follicular lymphoma in a Crohn's disease patient receiving anti-α4β7-integrin therapy: A case report

Keval Yerigeri, Ilia Buhtoiarov

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Keval Yerigeri, Internal Medicine-Pediatrics, Case Western Reserve University/MetroHealth, Cleveland, OH 44109, United States

Ilia Buhtoiarov, Pediatric Hematology/Oncology and Bone Marrow Transplantation, Cleveland Clinic Children's, Cleveland, OH 44106, United States

Corresponding author: Keval Yerigeri, MD, Doctor, Internal Medicine-Pediatrics, Case Western Reserve University/MetroHealth, 2500 Metrohealth Dr, Cleveland, OH 44109, United States. kyerigeri@metrohealth.org

Abstract

BACKGROUND

Patients with autoimmune conditions receiving immunosuppressants are at risk of non-Hodgkin lymphomas (NHL). Vedolizumab (anti-α4β7-integrin antibody), a treatment-of-choice for Crohn's disease (CD), reduces inflammatory lymphocyte trafficking into the intestinal mucosa. This effect is believed to be confined to the colon.

CASE SUMMARY

We report the case of a CD patient on vedolizumab for five years who developed pediatric-type follicular lymphoma. Work-up prior to therapy revealed a reduction in circulating T-lymphocytes and their suppressed response to mitogens. Rituximab, cyclophosphamide, vincristine, and prednisone chemo-immunotherapy resulted in durable lymphoma remission, and vedolizumab treatment was continued. While the patient's T-lymphocyte population and immunoglobulin production recovered, the T-lymphocyte mitogen response remained suppressed.

CONCLUSION

This patient's NHL may be linked to receiving anti-α4β7 therapy. Further research could be beneficial to determine if proactive surveillance for NHL and other systemic diseases is indicated in patients on vedolizumab.

Key Words: Pediatric-type follicular lymphoma; Crohn's disease; Vedolizumab; Immunosuppression; Non-Hodgkin lymphoma; Case report

Core Tip: The literature is inconclusive on the association between anti-α4β7-integrin therapy and oncogenesis. This case report highlights a young adult on chronic vedolizumab, a monoclonal antibody targeting α4β7-integrin, who develops pediatric-type follicular lymphoma. The patient recovered with rituximab, cyclophosphamide, vincristine, prednisone immunotherapy, but T-lymphocyte mitogen response remained suppressed.

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INTRODUCTION

Follicular lymphoma (FL) is one of the most common non-Hodgkin lymphomas (NHL) worldwide[1]. Pediatric-type FL (PTFL) was first identified as a distinct clinicopathological condition in the 4th edition of the World Health Organization classification of lymphoid neoplasms[2]. PTFL lacks the t(14;18) translocation characteristic of adult-type FL that leads to overexpression of the B-cell leukemia/lymphoma 2 (*BCL2*) oncogene. Instead, the most commonly affected gene in PTFL is *MAP2K1* on chromosome 15 encoding the serine/threonine kinase MEK1[3]. MEK1 plays a role in the RAS-MAPK signaling pathway and directs cell growth, differentiation, and apoptosis. PTFL commonly presents with localized disease (stage I or II) involving lymph nodes of the head and neck region. Hilar and mediastinal lymph nodes may also be involved. The adenopathy is typically painless and without mass effect on adjacent anatomical structures[4]. The disease course of PTFL is indolent and cure rate is high. Standard-of-care treatment approaches are surgical resection *versus* rituximab, cyclophosphamide, vincristine, and prednisone-like regimens for patients with advanced or unresectable conditions[5].

Patients with autoimmune conditions such as inflammatory bowel disease (IBD) and those treated with systemic immunosuppressants are at risk of developing lymphoid malignancies[6,7]. The incidence of such comorbidities remains low, and reports are limited to adult patient cohorts. Crohn's disease (CD) is a chronic IBD with increasing incidence in the United States over the past several decades. The pathogenesis of Crohn's is mediated by auto-reactive T-cells migrating into intestinal tissue, perpetuating inflammation and tissue necrosis. Targeted therapies have been developed to block inflammatory cell activation and migration in an organ-specific manner[8,9]. Vedolizumab is an anti-α4β7 integrin monoclonal antibody that inhibits interaction of T cells, monocytes, and dendritic cells with the mucosal addressin cell adhesion molecule-1 (MAdCAM-1) expressed on vascular endothelium. Paradoxically, ligation of α4β7 results in downregulation of genes controlling expression of innate immune receptors, chemokines, and their cognate receptors. Previous clinical studies suggest that vedolizumab does not have a systemic immunosuppressive effect and does not increase risk for malignancy[10,11].

We present a case of a 27-year-old male with CD who was diagnosed with PTFL five years into treatment with vedolizumab. Surprisingly, at the time of diagnosis, the patient had reduced numbers and suppressed function of circulating T cells, potentially contributing to a permissive immune environment for PTFL development. This case provides a useful addition to the existing knowledge of PTFL putative risk factors and vedolizumab systemic effects on the immune system.

CASE PRESENTATION

Chief complaints

A 27-year-old male presented with a painless mass in his right mandibular fossa.

History of present illness

Review of systems was negative. The patient denied constitutional B symptoms (fever, night sweats, weight loss), signs of gastrointestinal distress (*e.g.*, pain, nausea, vomiting, diarrhea), and oral pain or dysphagia.

History of past illness

Past medical history was pertinent for CD on long-term vedolizumab therapy.

Personal and family history

All other personal and family medical history was noncontributory.

Physical examination

Physical exam appreciated submandibular and upper cervical lymphadenopathy; there were no other concerning findings. Abdomen was soft, non-tender, non-distended, and without palpable masses; bowel sounds were present.

Laboratory examinations

Core-needle biopsy of the parotid mass demonstrated neoplastic infiltrate with a follicular pattern. The follicles did not have polarized germinal centers and immunophenotype. The neoplastic cells were intermediate-sized with scant cytoplasm and irregular nuclei. The neoplastic cells expressed CD10, CD20, BCL6, PAX5, and CD23; they did not express CD3, CD5, CD21, cyclin D1, BCL2, MUM1, or BCL2. Ki-67 stain was positive in approximately 70% of cells (Figure 1). Molecular studies identified a MAP2K1p.g128D mutation.

Imaging examinations

Computed tomography confirmed the mass within the parotid gland encasing the right-sided facial nerve.

FINAL DIAGNOSIS

The above pathomorphological and molecular features were consistent with FL, pediatric-type.

TREATMENT

Pre-therapy baseline immunologic testing revealed reduced absolute and relative levels of circulating $CD3^+CD4^+$ and $CD3^+CD8^+$ T-cells (Table 1). *In vitro* mitogen stimulation with phytohemagglutinin (PHA) and concanavalin A (ConA) also revealed suppressed proliferative response (Table 2). Of interest, while the peripheral blood B-cell population had a polyclonal pattern, the T-lymphocyte pool demonstrated expansion of two distinct T cell receptor (TCR)-rearranged monoclonal populations (Table 1), a phenomenon characteristic for autoimmune conditions such as IBD[12,13]. The patient was also positive for Epstein-Barr virus (EBV) viral capsid antigen (VCA) immunoglobulin (Ig)G [antibody index (AI) 7.1]; negative for EBV VCA IgM (0.2 AI); positive for EBV early antigen Ab (1.5 AI); and positive for EBV nuclear antigen Ab (7.8 AI), indicating chronic EBV viremia/reactivation. The parotid mass tissue was not stained for EBV, and EBV DNA quantification in blood was not performed.

The parotid gland was not amenable to surgical resection or local radiation therapy; therefore, 6 cycles of rituximab, cyclophosphamide, vincristine, and prednisone chemo-immunotherapy were administered concurrently with monthly vedolizumab. Complete remission for the lymphoma was successfully achieved with good control of CD symptoms (no recurrence or flares).

OUTCOME AND FOLLOW-UP

Repeat assessment at 12 mo off-therapy revealed ongoing complete remission of both PTFL and CD. Of note, one of the TCR-rearranged T-lymphocyte clones became undetectable (Table 1). Surprisingly, functional suppression of peripheral blood T-cells persisted. It remains unclear whether recurrent immune suppression at this clinical stage may only be attributed to continued vedolizumab therapy.

DISCUSSION

Lymphoproliferative disorders (including NHL) have long been recognized as complications for autoimmune inflammatory conditions[14]. Latent infection with EBV (as in this patient) or human herpesvirus 8 may also contribute to cancer genesis[15]. Vedolizumab, the monoclonal antibody against α4β7-integrin, uniquely inhibits interaction of T cells, monocytes, and dendritic cells with the vascular endothelium to reduce local inflammation for disease control[10]. While serious adverse events are reported in 41% of Crohn's patients, benign and malignant neoplasms are noted in only 6.8% of treated patients at an incidence rate of 20.8 per 1000 person-years[16]. Several other studies suggested that treatment with vedolizumab is rather safe and does not carry an excessive burden of new or recurrent malignancies[17].

However, recent insights into the mechanisms of vedolizumab's biologic activity suggest it may extend beyond inhibition of the α4β7-integrin interaction with MAdCAM-1. It appears that several important genes regulating immune effectors and mechanisms of their cross-talk *via* chemokines and cytokines [*e.g.*, CXC chemokine ligand (CXCL)9, CXCL10, FCGR3B, interleukin (IL)23A, IL17, interferon-γ] were down-regulated in IBD patients who achieved clinical remission with vedolizumab[18,19]. Additional findings suggest that the interaction of α4β7-integrin with MAdCAM-1 may serve as an alternative co-stimulatory pathway for T cells, triggering expression of genes encoding multiple cytokines (*e.g.*, IL-2, IL-3, IL-4, IL-8, IL-13, IL-17A, IL-17F, IL-22) in a similar fashion to CD28-mediated signaling. This potentially implies that inhibition of α4β7-integrin-MAdCAM-1 interaction results in down-regulation of T-cell function, although the argument requires further research[20].

Our patient was found to have decreased numbers of both $CD3^+CD4^+$ and $CD3^+CD8^+$ T-cells as well as a diminished response to ConA and PHA at the time of NHL diagnosis, *i.e.*, while receiving ongoing vedolizumab therapy. At the end of lymphoma chemo-immunotherapy and at 12 mo post-therapy, peripheral blood lymphocyte subset quantification demonstrated improvement of the absolute $CD3^+CD4^+$ and $CD3^+CD8^+$ T-cell counts; however, the proliferative response

Table 1 Peripheral blood lymphocyte subsets, and B- and T-lymphocyte clonality

Component	Ref range & units	Testing time point		
		Pre-Tx	EOTx	12 mo off Tx
$CD3^+$ T cell	60%-89%	43	72	52
$CD3^+$ T cell	958-2388 cells/uL	473	792	869
$CD3^+CD4^+$ T cell	34%-61%	27	39	32
$CD3^+CD4^+$ T cell	533-1674 cells/uL	304	425	526
$CD3^+CD8^+$ T cell	10%-41%	14	28	18
$CD3^+CD8^+$ T cell	175-958 cells/uL	151	303	293
$CD19^+$ B cell	5%-22%	20	0	23
$CD19^+$ B cell	75-660 cells/uL	224	0	385
NK cell	5%-25%	37	27	24
NK cell	102-565 cells/uL	406	297	394
CD4/CD8 ratio	1.10-3.25	2.02	1.40	1.79
B cell clonality				
IGH FR1		Nonclonal		Nonclonal
IGH FR2		Nonclonal		Nonclonal
IGH FR3		Nonclonal		Nonclonal
IGH DH1-6-J		Nonclonal		Nonclonal
IGK V-J		Nonclonal		Nonclonal
IGK V-Kde		Nonclonal		Nonclonal
TCR clonality				
TCRB A (V-J)		Nonclonal		Nonclonal
TCRB B (V-J)		Nonclonal		Nonclonal
TCRB C (V-J)		Clonal peak (180 bp)		Clonal peak (180 bp)
TCRG D		Clonal peak (189 bp)		Nonclonal

pre-Tx: Pre-treatment; EOTx: End of therapy; 12 mo off Tx: 12 mo off therapy anti-lymphoma therapy; NK: Natural killer; TCR: T cell receptor.

Table 2 Response of peripheral blood lymphocytes to *in vitro* mitogen stimulation

Component	Reference range & units	Testing time point	
		Pre-Tx	12 mo off Tx
Mitogen control	> 50 CPM	434	2297
Phytohemagglutinin	≥ 188800 CPM	173643	125008
Pokeweed mitogen	> 68549 CPM	77631	76811
Concanavalin A	> 81283 CPM	49198	92755

CPM: Counts per million; pre-TX: Pre-treatment; 12 mo off Tx: 12 mo off therapy anti-lymphoma therapy.

to ConA remained suppressed. At this phase of the clinical course, reduced response to the *in vitro* mitogen stimulation may be attributable to vedolizumab, although the suppressive effect of an auto-inflammatory state cannot be ruled out. Further research is necessary to discern if vedolizumab has responsible mechanisms. Regardless, the patient was able to recover his $CD19^+$ B-cell population, which was completely depleted by rituximab at the end of treatment, as well as maintain immunoglobulin production (Table 3). Surprisingly, one of the two clonally expanded populations of T-cells were not detectable following therapy completion, suggesting eradication by the anti-lymphoma chemotherapy.

Table 3 Immune globulin production before treatment, at the end of therapy and at 12 mo off therapy follow up

Component	Ref range & units	Testing time point		
		Pre-Tx	EOTx	12 mo off Tx
IgA	68-408 mg/dL	250	177	170
IgM	35-263 mg/dL	55	30	31
IgG	768-1632 mg/dL	1376	921	927
IgG Subclass 1	240-1118 mg/dL	763	-	503
IgG Subclass 2	124-549 mg/dL	371	-	335
IgG Subclass 3	21-134 mg/dL	162 (H)	-	64
IgG Subclass 4	1-123 mg/dL	64	-	40

Ig: Immunoglobulin; pre-Tx: Pre-treatment; EOTx: End of therapy; 12 mo off Tx: 12 mo off therapy anti-lymphoma therapy.

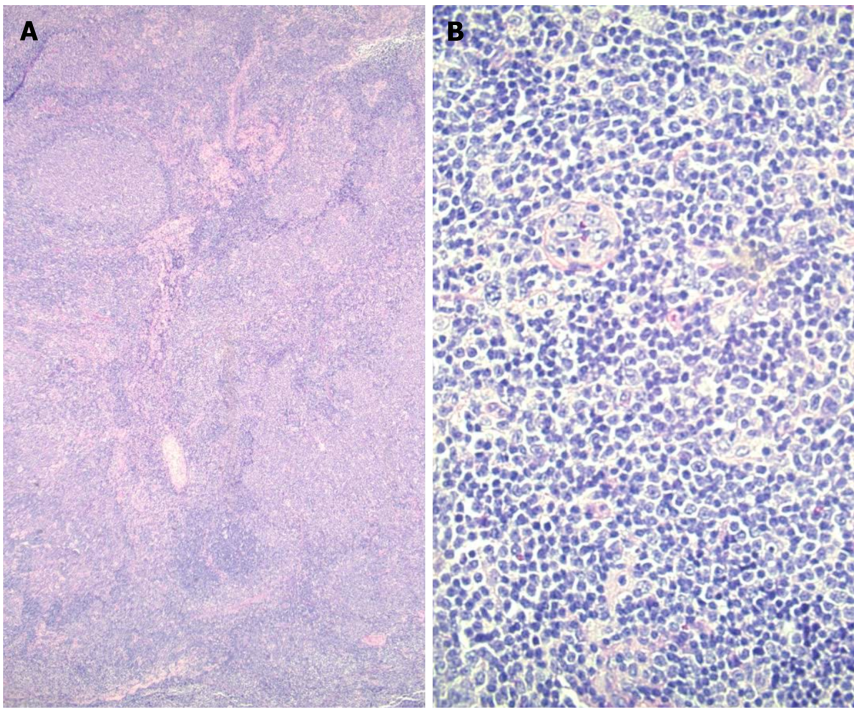

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Figure 1 Histopathological appearance and immunophenotype of pediatric-type follicular lymphoma. A and B: Typical appearance of pediatric-type follicular lymphoma at low- (A, 4 ×) and high-power (B, 40 ×) resolution of hematoxylin and eosin staining lymphoma tissue. Pattern of expression of CD10, CD20, BCL6 and Ki67 at 20 × resolution is also shown.

CONCLUSION

PTFL is a rare type of B-cell NHL. The clinical course is indolent despite a high proliferative index and blastoid histopathological features. Due to its rarity, predisposing factors have not been clearly postulated. The role of underlying immune suppression in PTFL pathogenesis has not been established. Nodal PTFL is believed to arise from B-lymphocytes in follicle germinal centers. Immune system workup and cytogenetic analysis in PTFL patients may help reveal an etiological correlation between the malignancy and immunomodulatory processes. Attention must also be paid to possible systemic consequences of vedolizumab therapy hitherto unreported in the literature.

FOOTNOTES

Author contributions: Buhtoiarov I was the patient's primary oncology attending, he provided the patient charts with informed consent, prepared images and tables for submission, and edited the manuscript; Yerigeri K prepared initial manuscript drafts and managed the submission and revisions.

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ORCID number: Keval Yerigeri 0009-0001-8963-0927; Ilia Buhtoiarov 0000-0001-5207-4616.

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